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Underestimated species diversity in *Zhangixalus* (Anura, Rhacophoridae) with a description of two cryptic species from southern China

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Abstract

It has been shown that species diversity of tree frogs in the genus *Zhangixalus* might have been underestimated. In this study, based on previously published data and newly collected samples from China, we constructed a phylogeny of the genus *Zhangixalus* using mitochondrial 12S rRNA-tRNA^{Val}-16S rRNA genes and revealed two novel lineages of *Zhangixalus* from southern China, one containing newly collected samples from Pingbian, Yunnan, and one containing samples from Chengbu, Hunan. Combining evidence from morphological comparison and species delimitation, we described these two lineages as two new species. *Zhangixalus daweishanensis* **sp. nov.** from Mt. Dawei, Pingbian is sister to *Z. dorsoviridis*, and it can be distinguished from congeners by body size medium (SVL 30.0–33.5 mm in males and 43.7–50.7 mm in females); heels not meeting when legs positioned at right angle to body; internal vocal sac; dorsum uniformly green; black blotches in axilla, groin, and posterior part of thigh; and throat yolk yellow. *Zhangixalus nanshanensis* **sp. nov.** from Nanshan National Park, Chengbu, Hunan, which has been confused with *Z. nigropunctatus*, is sister to the clade of *Z. lishuiensis* and *Z. zhoukaiyae* and is distinguishable from congeners by body size medium (SVL 28.1–36.7 mm in males and 45.2 mm in a female); dorsum uniformly green; vocal sac external; throat yellow with greyish brown blotches; outer metatarsal tubercle absent; heels not meeting when legs positioned at right angle to body; and black blotches in axilla, groin, and posterior part of thigh. In addition, we considered the samples of nominal *Z. dorsoviridis* from Son La, Vietnam represent a cryptic species, and more studies are required to unravel the taxonomy and distribution of *Z. dorsoviridis* and *Z. nigropunctatus* complexes, as the taxonomic status of some records of these two species in China also remains unclear. We supported that *Z. duboisi* and *Z. omeimontis* are distinct from each other and considered that *Z. zhoukaiyae* and *Z. lishuiensis* are two separate species.

Key Words

Cryptic species, phylogeny, species delimitation, *Zhangixalus dorsoviridis*, *Zhangixalus nigropunctatus*

Introduction

The genus *Zhangixalus* Li, Jiang, Ren & Jiang, 2019, which uses *Zhangixalus dugritei* (David, 1872) as the type species, was split from *Rhacophorus* Kuhl & Van Hasselt, 1822 *sensu lato*. It is characterized by medium-to-large body size, absence of dermal folds along limbs, absence of tarsal projections, terminal phalanges of fingers and toes Y-shaped, and usual green dorsal coloration (Jiang et al. 2019). The genus is distributed widely in eastern Asia and northern Indochina and currently contains 44 species (Frost 2024), of which 30 have been recorded in China (AmphibiaChina 2024).

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Studies in recent years have demonstrated that species diversity of *Zhangixalus* had been severely underestimated in the past owing to morphological conservation, and new species have been constantly discovered with the use of molecular data (e.g., Yu et al. 2019a; Nguyen et al. 2020, 2024; Ninh et al. 2020; Brakels et al. 2023; Gonggoli et al. 2024; Nguyen et al. 2024; Pan et al. 2024). Among those new species discovered recently, a few of them were newly discovered with narrow distribution (e.g., Nguyen et al. 2020; Ninh et al. 2020; Brakels et al. 2023), and most were split from species previously considered to be distributed widely or were confused with known species, which mainly involves the taxonomic changes in the *Z. nigropunctatus* (Liu, Hu & Yang, 1962) and *Z. dorsoviridis* (Bourret, 1937) complexes. *Zhangixalus nigropunctatus* was originally described from Weining, western Guizhou, China (Liu et al. 1962) and was once recorded widely from Yunnan, Guizhou, Hunan, and Anhui with discontinuous distribution in China (Fei 1999; Fei et al. 2012). However, the samples of nominal *Z. nigropunctatus* from Anhui were recovered as sister to *Z. dorsoviridis* and described as *Z. zhoukaiyae* (Pan, Zhang & Zhang, 2017) by Pan et al. (2017), the samples of nominal *Z. nigropunctatus* from Pingbian and Jinping of Yunnan did not cluster together with topotypes of *Z. nigropunctatus* (Yu et al. 2009; Li et al. 2012a) and were moved into *Z. dorsoviridis* (Li et al. 2012a), and the samples of nominal *Z. nigropunctatus* from Xinping and Longling of Yunnan represent a cryptic species (*Z. yunnanensis* Pan, Hou, Yu & Liu, 2024; Pan et al. 2024), implying that taxonomic status of other records of this species (e.g. Hunan) also needs further investigation (Pan et al. 2024).

Moreover, it has been revealed that some species with similar external morphology occurred sympatrically, which could easily lead to misidentification and therefore underestimation of species diversity. For example, the specimen ROM 38011 was collected from the type locality of *Z. dorsoviridis* (Sa Pa, Vietnam) and was originally identified as *Z. dorsoviridis* (Orlov et al. 2001). However, this specimen differs from *Z. dorsoviridis* by having a dark throat, and phylogenetically it is not related to true *Z. dorsoviridis* (Li et al. 2012a; Mo et al. 2016; Liu et al. 2017; Pan et al. 2024). Although it was once moved to *Z. nigropunctatus* by Li et al. (2012a), it is actually neither *Z. dorsoviridis* nor *Z. nigropunctatus* (Pan et al. 2024), but belongs to *Z. thaoae* Nguyen, Nguyen, Ninh, Le, Bui, Orlov, Hoang & Ziegler, 2024, a new species described recently by Nguyen et al. (2024). In addition, based on Pan et al. (2024), the taxonomic status of the sample of nominal *Z. dorsoviridis* from Son La, Vietnam (VNMN 4099) is also unclear. Therefore, the actual diversity of *Zhangixalus* probably remains underestimated due to the common occurrence of endemism and narrow-ranged species (Dufresnes and Litvinchuk 2022).

During our recent field surveys in southern Yunnan and southwestern Hunan, China (Fig. 1), some *Zhangixalus* specimens resembling *Z. dorsoviridis*, *Z. nigropunctatus*, and *Z. thaoae* were collected from Pingbian and Chengbu Counties. Considering the underestimated species diversity of *Zhangixalus* and the taxonomic history of *Z. dorsoviridis* and *Z. nigropunctatus*, we sequenced these newly collected treefrogs to confirm their identity. Our phylogenetic analyses revealed that these specimens form two distinct lineages, one containing specimens

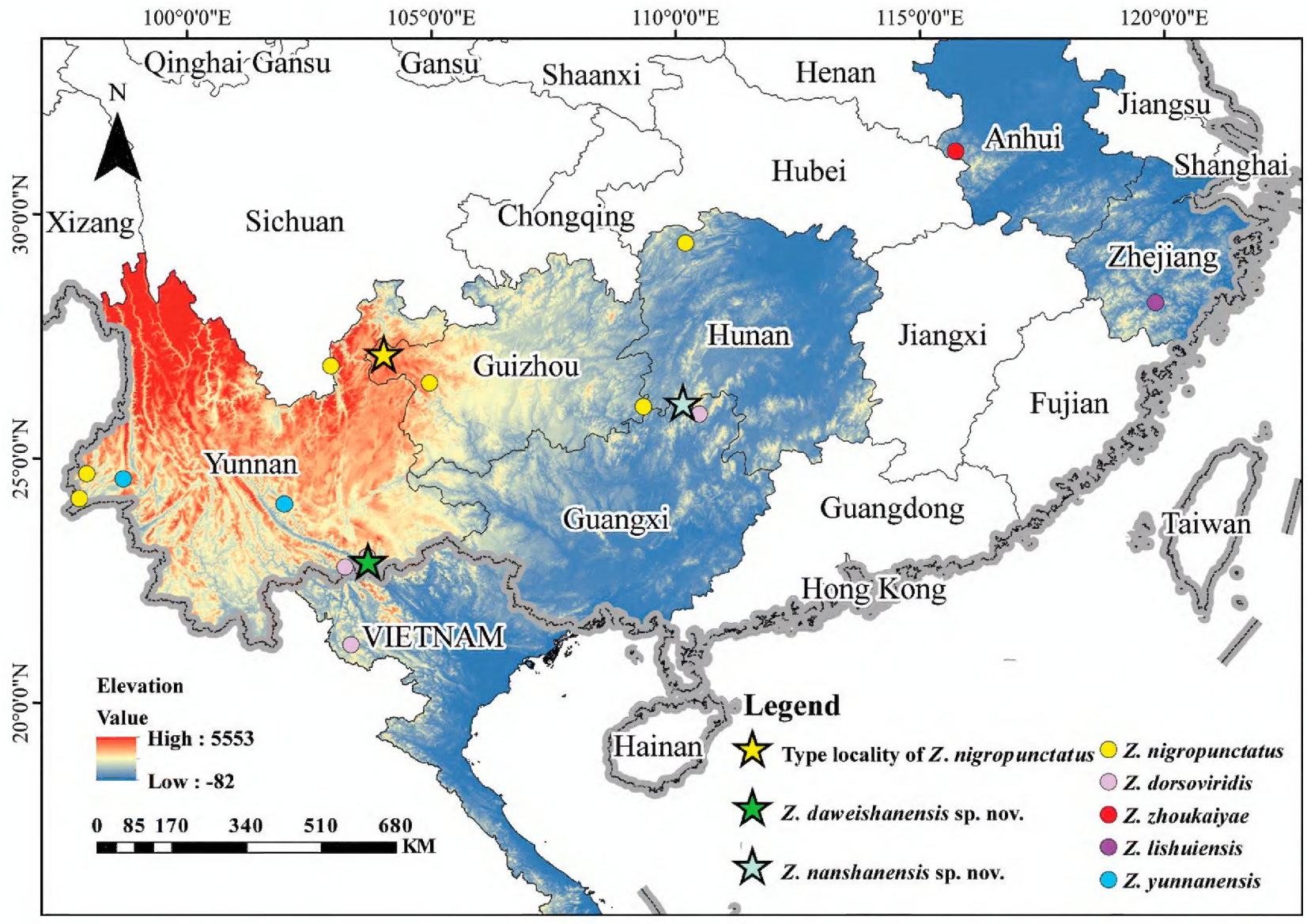

Figure 1. Known distribution sites of *Z. daweishanensis* sp. nov. and *Z. nanshanensis* sp. nov. and related species. The stars represent the type localities of the two new species and *Z. nigropunctatus*. The map was generated using ArcMap v.10.2 (ESRI Inc.).

newly collected from Pingbian, Yunnan, and one containing specimens from Chengbu, Hunan. Furthermore, our morphological examination suggested that these two lineages differ from known members of *Zhangixalus* by a series of characters. Herein, we officially describe them as two new species of *Zhangixalus*.

Materials and methods

Sampling

This study was carried out in accordance with the ethical guidelines issued by the Ethics Committee of Guangxi Normal University. In Yunnan, China, field surveys were conducted on 9 March and 3 May 2021 at Pingbian County, and a total of seven treefrog specimens of *Zhangixalus* were collected during the surveys. In Hunan, China, field surveys were conducted on 5 April 2024 at Chengbu County, and a total of eight treefrog specimens of *Zhangixalus* were collected during the surveys. Specimens were photographed, euthanized, fixed, and then stored in 75% ethanol. Liver tissues were preserved in 99% ethanol. Specimens were deposited at Guangxi Normal University (GXNU).

Phylogenetic analysis and species delimitation

Total genomic DNA was extracted from liver tissue samples that were stored in 99% ethanol. A fragment encoding mitochondrial 12S rRNA, $tRNA^{val}$, and 16S rRNA genes was amplified and sequenced using the primers and protocols of Yu et al. (2019a). 12 samples (seven from Pingbian and five from Chengbu) were newly sequenced, and all new sequences have been deposited in GenBank under Accession PV010194–PV010202 and PQ998508–PQ998510 (Table 1). Additionally, 53 homologous sequences of other *Zhangixalus* species and outgroups were obtained from GenBank (Table 1). *Theloderma albopunctatum* (Liu & Hu, 1962), *Rhacophorus rhodopus* (Liu & Hu, 1960), and *Leptomantis gauni* (Inger, 1966) were included in the data as outgroups.

Sequences were aligned using MUSCLE with default parameters in MEGA v.XI (Tamura et al. 2021). Uncorrected genetic *p*-distances (using 16S rRNA sequences) between species were calculated in MEGA v.XI. The best substitution model was selected using the corrected Akaike Information Criterion (AICc) in jModelTest v.2.1.10 (Darriba et al. 2012). Bayesian Inference was performed in MrBayes v.3.2.7 (Ronquist et al. 2012) under the selected substitution model (GTR + I + G). Two runs were performed simultaneously with four Markov chains starting from a random tree. The chains were run for 5,000,000 generations and sampled every 100 generations. The first 25% of the sampled tree was discarded as burn-in after the standard deviation of split frequencies of the two runs was less than 0.01. The remaining trees were then used to create a consensus tree and to estimate Bayesian posterior probabilities (BPPs). In addition, a Maximum Likelihood (ML) analysis was conducted in RaxmlGUI v. 8.2 (Stamatakis 2014) with 1000 rapid bootstrap replicates. The node was considered strongly supported with BPP $\geq$ 0.95 and bootstrap value $\geq$ 70 (Leaché and Reeder 2002).

We used Assemble Species by Automatic Partitioning (ASAP) for species delimitation. For this method, we used the simple distance (*p*-distance) model, and the partition with the lowest ASAP P-score was the best partition (Puillandre et al. 2021).

Morphology

Morphometric data were taken using electronic digital calipers to the nearest 0.1 mm. Morphological terminology followed (Yu et al. 2019a). Measurements included: snout-vent length (SVL, from tip of snout to vent); head length (HL, from tip of snout to rear of jaws); head width (HW, width of head at its widest point); snout length (SL, from tip of snout to anterior corner of eye); internarial distance (IND, distance between nares); interorbital distance (IOD, minimum distance between upper eyelids); upper eyelid width (UEW, maximum width of upper eyelid); eye diameter (ED, diameter of exposed portion of eyeball); distance between nostril and eye (DNE, from nostril to anterior border of eye); tympanum diameter (TD, the greater of tympanum vertical and horizontal diameters); forearm and hand length (FHL, from elbow to tip of third finger); tibia length (TL, distance from knee to heel); foot length (FL, from proximal end of inner metatarsal tubercle to tip of fourth toe); and length of foot and tarsus (TFL, from tibiotarsal joint to tip of fourth toe). Webbing formula followed (Myers and Duellman 1982). Comparative morphological data of other *Zhangixalus* species were taken from their original descriptions or re-descriptions (Boulenger 1892; Stejneger 1907; Smith 1924a; Inger 1947; Liu et al. 1962; Inger 1966; Lue et al. 1994, 1995; He 1999; Orlov et al. 2001; Ohler et al. 2000; Harvey et al. 2002; Matsui and Panha 2006; Rao et al. 2006; Bordoloi et al. 2007; Chou et al. 2007; Fei et al. 2009, 2010; Jiang et al. 2016; Zhang et al. 2011; Fei et al. 2012; Li et al. 2012b; Mo et al. 2016; Liu et al. 2017; Pan et al. 2017; Chen et al. 2018; Yu et al. 2019a; Nguyen et al. 2020; Ninh et al. 2020; Brakels et al. 2023; Gonggoli et al. 2024; Lue et al. 2024; Nguyen et al. 2024; Pan et al. 2024). Multivariate principal component analysis (PCA) was conducted in SPSS v.17.0 (SPSS Inc.), based on the correlation matrix of size-standardized measurements (all measurements divided by SVL) of adult males. Scatter plots of the scores of the first three factors of PCA were used to examine the differentiation between the new species and its closest relatives revealed by phylogenetic analyses. Differences in quantitative characters of adult males between these three species were also evaluated with t-tests in SPSS. In these analyses, Levene's test was also performed for each character to test for equality of variances.

Table 1. Species used for phylogenetic analyses in this study.

Species	Voucher No.	Locality	Accession No.
Leptomantis gauni	FMNH273928	Sarawak, Malaysia	JX219456
Rhacophorus rhodopus	SCUM 060692L	Mengyang, Yunnan, China	EU215531
Theloderma albopunctatum	ROM 30246	Vietnam	AF458148
Zhangixalus arboreus	TTU–R–11748	Japan	AF458142
Z. burmanus	SCUM 060614L	Mt. Gaoligong, Yunnan, China	EU215537
Z. chenfui	SCUM 060404L	Mt. Omei, Sichuan, China	EU215534
Z. daweishanensis sp. nov.	GXNU YU000392	Pingbian, Yunnan, China	PQ998508
Z. daweishanensis sp. nov.	GXNU YU000393	Pingbian, Yunnan, China	PV010195
Z. daweishanensis sp. nov.	GXNU YU000394	Pingbian, Yunnan, China	PV010196
Z. daweishanensis sp. nov.	GXNU YU000395	Pingbian, Yunnan, China	PQ998507
Z. daweishanensis sp. nov.	GXNU YU000396	Pingbian, Yunnan, China	PV010197
Z. daweishanensis sp. nov.	KIZL040279	Pingbian, Yunnan, China	PQ998510
Z. daweishanensis sp. nov.	KIZL040278	Pingbian, Yunnan, China	PQ998509
Z. dennysi	ROM 30249	Vietnam	AF458139
Z. dorsoviridis	ROM 38015	Sa Pa, Lao Cai, Vietnam	JX219423
Z. dorsoviridis	Rao060821200	Jinping, Yunnan, China	JX219424
Z. dorsoviridis	YN080446	Pingbian, Yunnan, China	JX219425
Z. dorsoviridis	Rao060821199	Pingbian, Yunnan, China	JX219426
Z. dorsoviridis	KIZ060821287	Jinping, Yunnan, China	EF564491
Z. duboisi	VNMN:4103	Lao Cai, Vietnam	LC010581
Z. duboisi	VNMN:4104	Lao Cai, Vietnam	LC010582
Z. dugritei	SCUM 051001L	Baoxing, Sichuan, China	EU215541
Z. dulitensis	BORNEENSIS09087	Borneo, Malaysia	AB847123
Z. feae	SCUM 050642W	Hekou, Yunnan, China	EU215544
Z. franki	VNMN 011687	Ha Giang, Vietnam	LC548746
Z. hongchibaensis	CIB 097687	Wuxi, Chongqing, China	JN688883
Z. hui	Li01	Zhaojue, Sichuan, China	JN688878
Z. hungfuensis	SCUM 060425L	Wenchuan, Sichuan, China	EU215538
Z. jodiae	VNMN 07122	Vietnam	LC545595
Z. lishuiensis	YPX47792	Lishui, Zhejiang, China	KY653720
Z. lishuiensis	GXNU YU000510	Lishui, Zhejiang, China	PQ901907
Z. lishuiensis	GXNU YU000509	Lishui, Zhejiang, China	PQ901906
Z. melanoleucus	BEI 01010	Phou Samsoum Mt., Xiengkhoang, Laos	OQ305233
Z. melanoleucus	ZMMU A–7781	Phou Samsoum Mt., Xiengkhoang, Laos	OQ305234
Z. melanoleucus	BEI 01011	Phou Samsoum Mt., Xiengkhoang, Laos	OQ305235
Z. melanoleucus	AUP 02507	Phou Samsoum Mt., Xiengkhoang, Laos	OQ305236
Z. minimus	KIZ 061214YP	Mt. Dayao, Guangxi, China	EU215539
Z. moltrechti	SCUM 061106L	Lianhuachi, Taiwan, China	EU215543
Z. nanshanensis sp. nov.	GXNU YU000771	Chengbu, Hunan, China	PV010198
Z. nanshanensis sp. nov.	GXNU YU000772	Chengbu, Hunan, China	PV010199
Z. nanshanensis sp. nov.	GXNU YU000773	Chengbu, Hunan, China	PV010200
Z. nanshanensis sp. nov.	GXNU YU000774	Chengbu, Hunan, China	PV010201
Z. nanshanensis sp. nov.	GXNU YU000776	Chengbu, Hunan, China	PV010202
Z. nigropunctatus	EU924623	Weining, Guizhou, China	EU924623
Z. nigropunctatus	GZ070658	Weining, Guizhou, China	JX219430
Z. nigropunctatus	SCUM 070657L	Weining, Guizhou, China	EU215533
Z. nigropunctatus	GXNU YU000361	Weining, Guizhou, China	PP187265
Z. nigropunctatus	GXNU YU000362	Weining, Guizhou, China	PP187266
Z. nigropunctatus	GXNU YU000363	Weining, Guizhou, China	PP187267
Z. omeimontis	SCUM 061104L	Pingbian, Yunnan, China	EU215536
Z. omeimontis	SCUM 0606137L	Pengxian, Sichuan, China	EU215535
Z. pachyproctus	KIZ090148	Puer, Yunnan, China	MN613222
Z. pinglongensis	NHMG201002011	Shiwandashan, Guangxi, China	KU170684
Z. puerensis	SCUM 060649L	Puer, Yunnan, China	EU215542
Z. schlegelii	–	Hiroshima, Japan	AB202078
Z. smaragdinus	KIZ 20160298	Yingjiang, Yunnan, China	MN613219
Z. thaoae	IEBR A 5136	Lao Cai, Vietnam	LC762092
Z. thaoae	IEBR A 5137	Lao Cai, Vietnam	LC762093
Z. thaoae	IEBR A 5138	Lao Cai, Vietnam	LC762094
Z. thaoae	ROM 38011	Sa Pa, Lao Cai, Vietnam	JX219427
Z. yaoshanensis	NHMG150408	Jinxiu, Guangxi, China	MG322122
Z. yunnanensis	GXNU YU20160269	Xinping, Yunnan, China	PV010194
Z. yunnanensis	GXNU YU20160268	Xinping, Yunnan, China	PP187269
Z. yunnanensis	GXNU YU20160267	Xinping, Yunnan, China	PP187270
Z. zhoukaiyae	AHU–RhaDB–120428	Jinzhai, Anhui, China	KU601502
Z. zhoukaiyae	GXNU YU05293	Jinzhai, Anhui, China	PQ901908
Zhangixalus sp.	VNMN:4099	Ta Xua, Son La, Vietnam	LC010577

Results

Phylogenetic relationship

The obtained sequence alignment was 1972 bp. Phylogenetic analyses revealed that the specimens newly collected from Mt. Dawei, Pingbian, Yunnan (GXNU YU000392–396, KIZL040278–279) form a novel clade. This clade was recovered as sister to the clade consisting of *Z. dorsoviridis* from the type locality (Sa Pa, Vietnam), Jinping, and Pingbian with strong support (100% for BI and 93% for ML) (Fig. 2), meaning that there is a cryptic lineage living in sympatry with *Z. dorsoviridis* at Mt. Dawei, Pingbian, Yunnan, China. In addition, the specimens collected from Nanshan National Park, Chengbu, Hunan (GXNU YU000771–774, YU000776) also formed a novel clade, and it was sister to the clade of *Z. lishuiensis* (Liu, Wang & Jiang, 2017) and *Z. zhoukaiyae*; then the three together were sister to the clade formed by *Z. dorsoviridis* and the cryptic lineage in Pingbian with strong support (100% for BI and ML) (Fig. 2).

The average genetic distance within the genus *Zhangixalus* ranges from 0.58% between *Z. hui* (Liu, 1945) and *Z. dugritei* (David, 1872) to 15.67% between *Z. jodiae* Nguyen, Ninh, Orlov, Nguyen & Ziegler, 2020, and *Z. dulitensis* (Boulenger, 1892). The 16S *p*-distances between the novel clade consisting of Pingbian specimens and the other *Zhangixalus* lineages included in this study ranged from 2.02% (vs. *Z. dorsoviridis*) to 10.25% (vs. *Z. dulitensis*), and the genetic distances between the novel lineage from Chengbu, Hunan, and other *Zhangixalus* species ranged from 1.93% (vs. *Z. lishuiensis*) to 12.07% (vs. *Z. dulitensis*) in 16S rRNA sequences (Suppl. material 2).

The best partition (score = 2.0) obtained by the ASAP analysis assigned all *Zhangixalus* samples used in this study into 31 species, two of which correspond to the clade of samples from Chengbu, Hunan, and the clade of the newly collected samples from Pingbian, Yunnan, respectively. *Zhangixalus dugritei* and *Z. hui* were assigned into one; the sample of nominal *Z. dorsoviridis* from Son La, Vietnam (VNMN 4099) was assigned into one with *Z. jodiae*, and other candidate species completely correspond to other known species used in this study (Fig. 3).

Morphometric analysis

Morphometric data are summarized in Suppl. material 1. The novel lineage from Mt. Dawei can be distinguished from *Z. dorsoviridis* by a smaller internarial distance and tympanum (Tables 2, 3), ventral surface of the thigh that is orange-yellow during the breeding season (vs. bright orange-red), heels not meeting when legs positioned at right angle to body (vs. overlapping), and other characters (see diagnosis below).

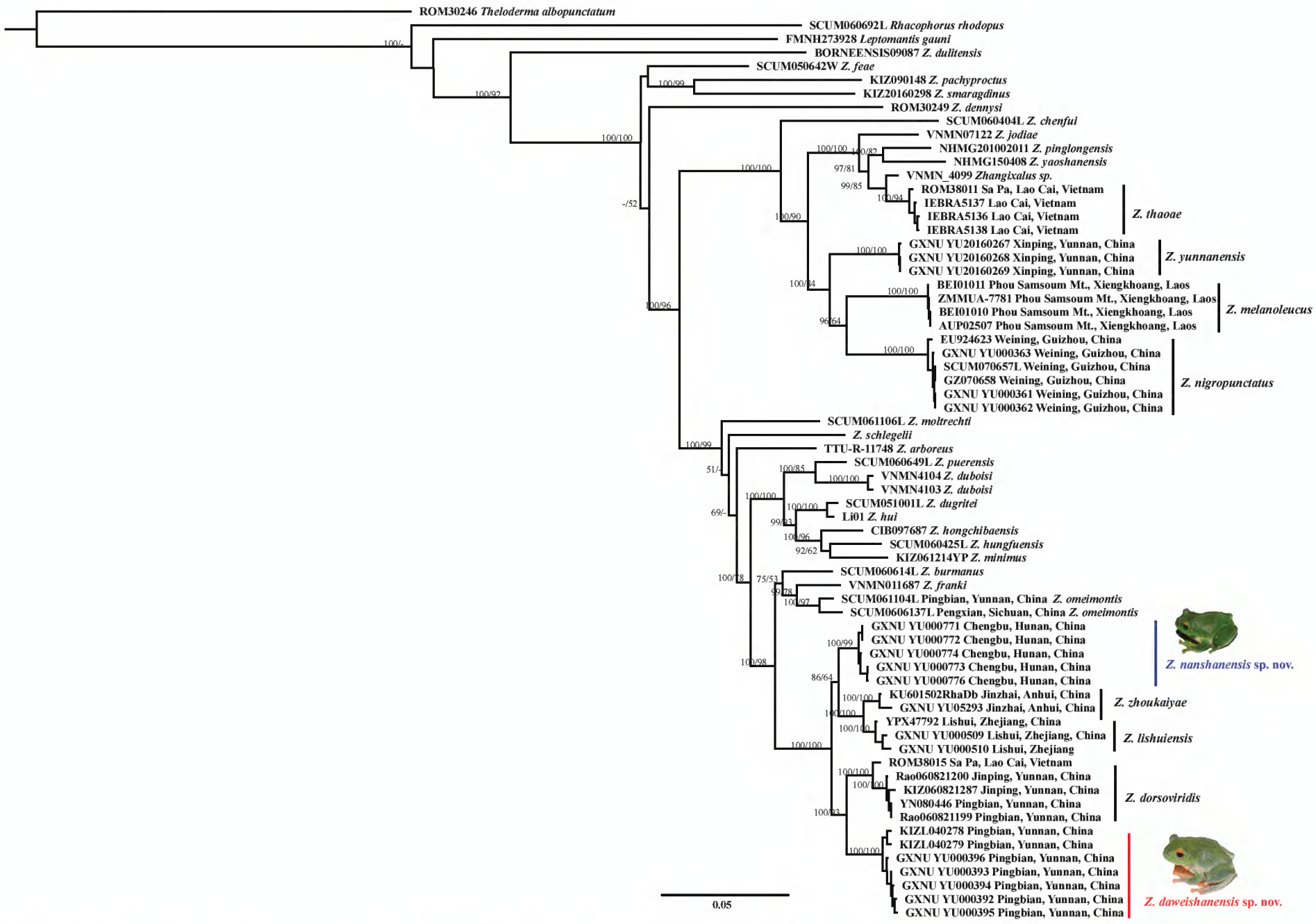

Figure 2. Bayesian phylogram of *Zhangixalus* inferred from mitochondrial 12S–tRNA^{Val}–16S sequences. Numbers above and below branches are Bayesian posterior probabilities and ML bootstrap values (only values above 50% are shown), respectively.

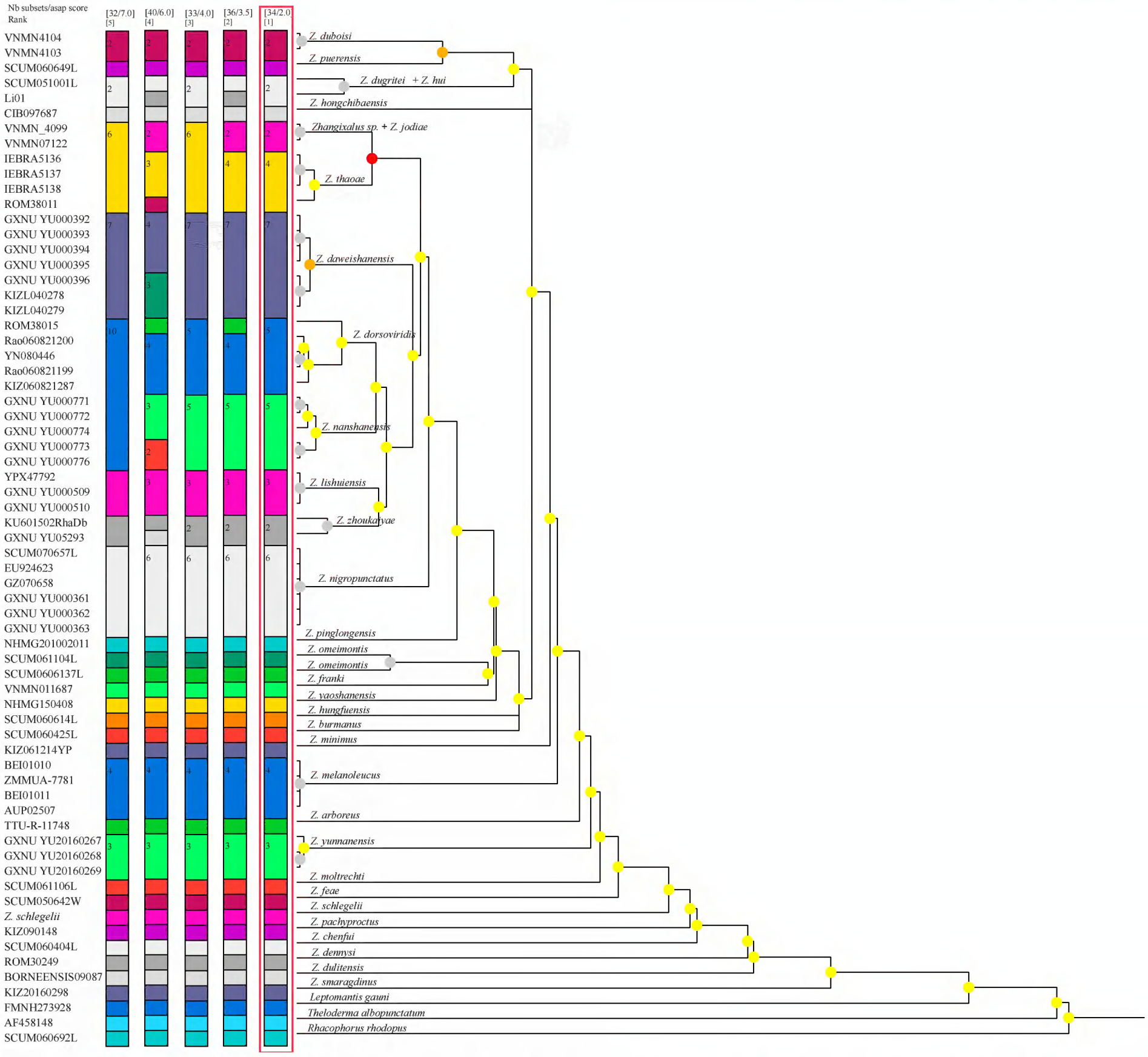

Figure 3. ASAP species delimitation based on 16S rRNA sequences. The best partition with the lowest score is highlighted with a red frame.

Table 2. Measurements (mm) of *Z. daweishanensis* sp. nov. (n = 7) and *Z. dorsoviridis* (n = 11; from Orlov et al. 2001).

Character	*Z. daweishanensis* sp. nov.				*Z. dorsoviridis*			
	male (n = 3)		female (n = 4)		male (n = 9)		female (n = 2)	
	range	Mean ± S.D	range	Mean ± S.D	range	Mean ± S.D	range	Mean ± S.D
SVL	32.0–33.5	32.5 ± 0.8	43.7–50.7	46.0 ± 3.2	31.37–42.43	37.18 ± 3.91	37.91–42.83	40.19 ± 3.73
HL	11.3–11.7	11.5 ± 0.2	14.4–15.6	14.8 ± 0.5	11.24–15.71	13.22 ± 1.51	12.51–14.14	13.13 ± 1.20
HW	12.3–12.8	12.5 ± 0.3	15.9–17.3	16.3 ± 0.6	11.05–15.29	13.51 ± 1.51	14.53–15.06	14.78 ± 1.22
SL	5.4–5.9	5.7 ± 0.2	7.0–8.1	7.6 ± 0.4				
IND	4.1	4.1 ± 0.0	5.2–5.7	5.4 ± 0.2				
IOD	4.3–4.5	4.4 ± 0.1	4.8–5.6	5.3 ± 0.4	6.88–9.05	7.67 ± 0.80	8.80–9.58	9.16 ± 1.46
UEW	2.2–2.5	2.4 ± 0.2	2.9–3.3	3.1 ± 0.3				
ED	4.1–4.4	4.3 ± 0.1	4.3–5.3	4.9 ± 0.4	3.40–4.66	4.05 ± 0.42	3.90–4.64	4.27 ± 0.52
TD	2.0–2.2	2.1 ± 0.1	2.6–3.3	3.1 ± 0.3	1.69–3.15	2.30 ± 0.51	2.37–2.40	2.39 ± 0.02
DNE	2.2–2.3	2.2 ± 0.1	3.1–3.5	3.4 ± 0.2				
FHL	16.6–17.1	16.8 ± 0.3	21.9–24.1	23.3 ± 1.0				
TL	14.2–20.6	18.3 ± 3.5	13.6–20.8	16.9 ± 3.3	10.85–19.12	15.66 ± 2.66	13.70–17.96	15.83 ± 3.00
TFL	21.7–31.8	27.8 ± 5.4	21.8–32.1	26.4 ± 5.0				
FL	14.7–21.4	18.8 ± 3.5	14.2–21.7	17.6 ± 3.6	12.63–17.43	15.41 ± 1.83	17.20–19.50	18.34 ± 1.68

Table 3. Summary statistics of male specimens (mean ± standard deviation) and results of the *t*–test between the *Z. daweishanensis* sp. nov. (n = 3) and *Z. dorsoviridis* (n = 5) from Pingbian, Yunnan. The *t*–test was performed on the size–adjusted data, except SVL. * = $p < 0.05$, ** = $p < 0.01$.

Character	Mean ± SD(n = 3)	Mean ± SD(n = 5)	Levene's test		t–test	
	Z. daweishanensis	*Z. dorsoviridis*	F	P–value	t	P–value
SVL	32.57 ± 0.823	37.04 ± 2.625	1.541	0.261	-2.788	0.0320*
HL	0.353 ± 0.013	0.372 ± 0.017	0.792	0.408	-1.603	0.1600
HW	0.385 ± 0.018	0.386 ± 0.008	2.382	0.174	-0.136	0.8960
SL	0.174 ± 0.006	0.176 ± 0.010	1.827	0.225	-0.252	0.8100
IND	0.126 ± 0.004	0.143 ± 0.007	4.913	0.069	-3.78	0.0090**
IOD	0.137 ± 0.007	0.138 ± 0.008	0.192	0.677	-0.252	0.8090
ED	0.131 ± 0.007	0.121 ± 0.011	1.007	0.354	1.441	0.2000
TD	0.066 ± 0.004	0.081 ± 0.003	0.566	0.48	-5.62	0.0010**
FHL	0.516 ± 0.020	0.517 ± 0.029	0.584	0.474	-0.05	0.9620
TL	0.561 ± 0.100	0.422 ± 0.031	9.512	0.022	2.346	0.1300
TFL	0.853 ± 0.151	0.781 ± 0.236	5.873	0.052	0.466	0.6570
FL	0.575 ± 0.099	0.437 ± 0.013	12.224	0.013	2.344	0.1320

We retained the first two principal components that accounted for 74.32% of the total variance (Table 4). Loadings for PC1, which accounted for 40.48% of the total variance, were most heavily loaded on internarial distance (IND), and Loadings for PC2, which accounted for 33.84%, were most heavily loaded on snout length (SL). Differentiation was found along the PC1 axis between the newly collected specimens from Pingbian and *Z. dorsoviridis* (Fig. 4).

For the cryptic lineage from Nanshan National Park, the measurement DNE was not included in PCA analysis because it was not available for *Z. lishuiensis* and *Z. zhoukaiyae*. We retained the three principal components that accounted for 75.25% of the total variance (Table 5). Loadings for PC1, which accounted for 36.71% of the total variance, were most heavily loaded on foot length (FL). Loadings for PC2, which accounted for 21.63% of the total variance, were most heavily loaded on forearm and hand length (FHL), and Loadings for PC3, which accounted for 16.91%, were most heavily loaded on head length (HL). Differentiation was found along both the PC1 and PC3 axes between the specimens from Nanshan National Park and *Z. lishuiensis* from the type locality, and differentiation was also found along the PC3 axis between the specimens from Nanshan National Park and *Z. zhoukaiyae*. The results of PCA analysis revealed distinct morphometric differences in head length between the specimens from Nanshan National Park and *Z. lishuiensis*, as well as in head length between the specimens from Nanshan National Park and *Z. zhoukaiyae* (Fig. 5). Moreover, the t-tests demonstrated that male specimens from Nanshan National Park differed significantly ($p < 0.05$) from male topotypes of *Z. zhoukaiyae* in HW, IOD, UEW, and TD (Table 6) and differed significantly ($p < 0.05$) from *Z. nigropunctatus* in HW, UEW, ED, TD, TL, and TFL (Suppl. material 4: table S4). Additionally, the new lineage is distinguishable from its congeners by body size and the combination of texture and coloration pattern.

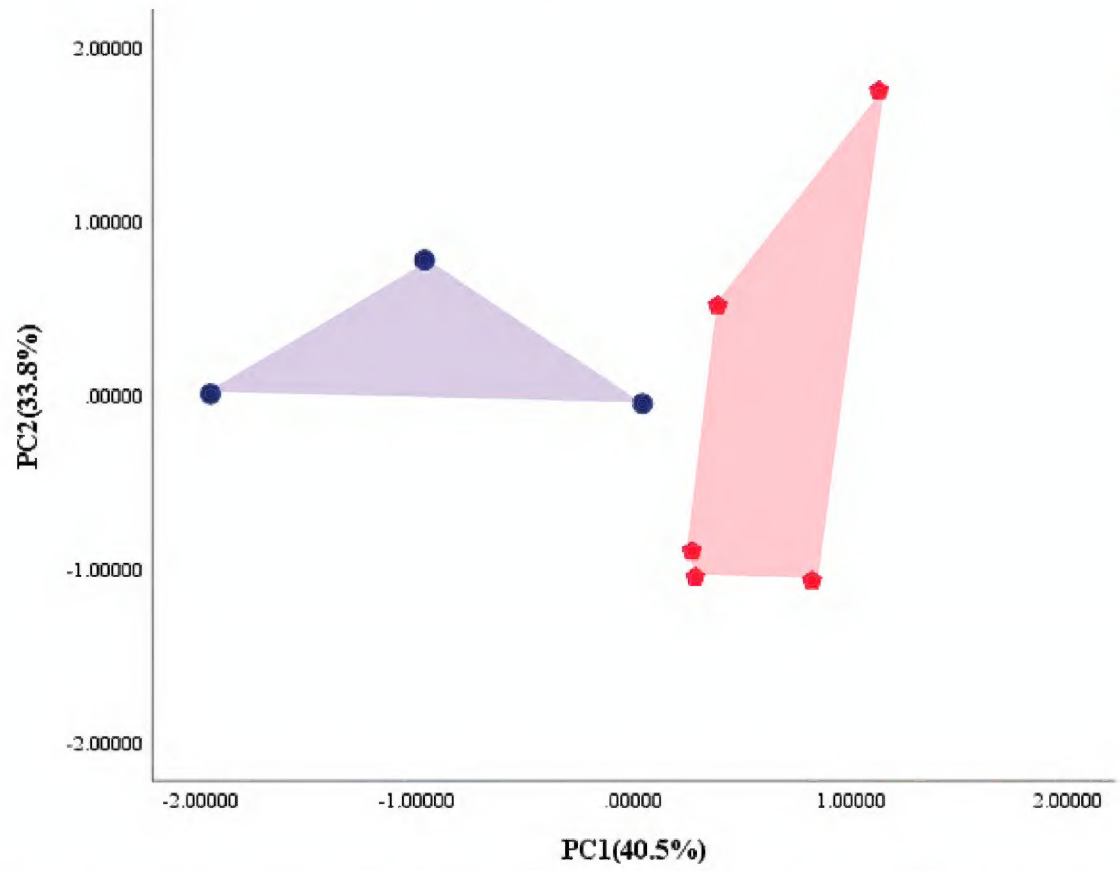

Figure 4. Scatterplot of principal components 1 and 2 of size–adjusted male morphometric data of *Z. daweishanensis* sp. nov. and *Z. dorsoviridis* from Pingbian, Yunnan, China.

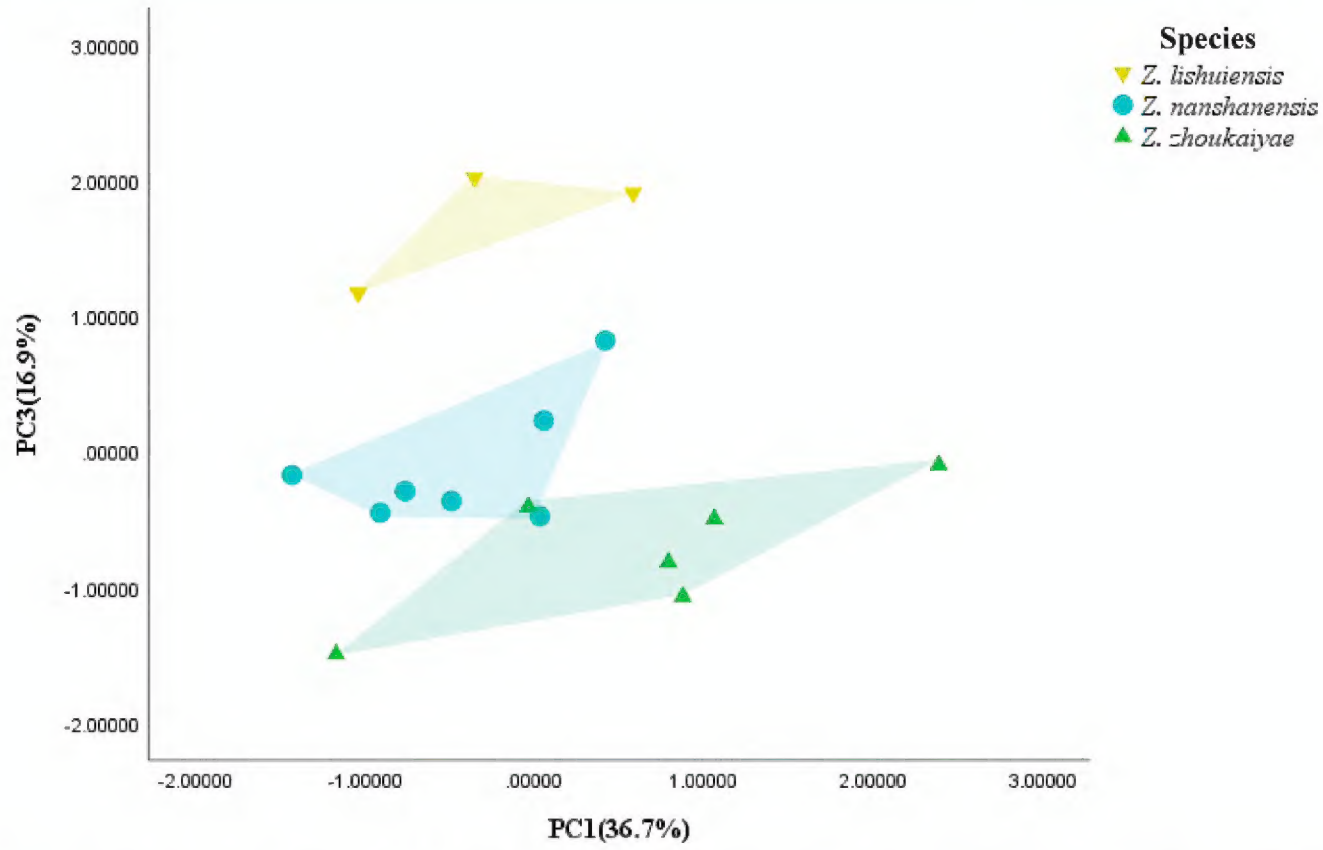

Figure 5. Scatterplot of principal components 1 and 3 of size–adjusted male morphometric data of *Z. nanshanensis* sp. nov., *Z. lishuiensis*, and *Z. zhoukaiyae*.

Table 4. Factor loadings of the first two principal components of 11 size–adjusted male morphometric characteristics of *Z. daweishanensis* sp. nov. and *Z. dorsoviridis* from Pingbian, Yunnan.

Character	PC1	PC2
Eigenvalue	4.453	3.722
% variation	40.482	33.838
HL	0.813	0.364
HW	0.630	0.397
SL	0.052	**-0.958**
IND	**0.915**	0.015
IOD	0.432	0.623
ED	-0.320	-0.416
TD	0.776	-0.343
FHL	0.414	0.788
TL	-0.831	0.463
TFL	-0.227	0.898
FL	-0.868	0.441

Table 5. Factor loadings of the first three principal components of 12 size–adjusted male morphometric characteristics of *Z. nanshanensis* sp. nov., *Z. lishuiensis*, and *Z. zhoukaiyae*.

Character	PC1	PC2	PC3
Eigenvalue	4.405	2.595	2.029
% variation	36.710	21.627	16.910
HL	-0.083	-0.285	**0.906**
HW	0.733	-0.423	0.308
SL	0.058	0.645	0.368
IND	0.513	-0.405	0.456
IOD	0.705	-0.395	-0.465
UEW	0.594	-0.568	-0.456
ED	0.636	0.413	0.327
TD	0.381	-0.510	0.328
FHL	0.318	**0.722**	0.057
TL	0.851	0.297	0.094
TFL	0.755	0.432	-0.345
FL	**0.895**	0.219	0.026

Therefore, on the basis of the above molecular and morphological evidence, we officially describe these two cryptic lineages as two new species.

Taxonomic account

Zhangixalus daweishanensis sp. nov.

https://zoobank.org/F65518E3-F6C4-4FFF-9832-0BB28FF88B00

Figs 6–9

Daweishan tree frog/Dà Weī Shān Shù wā (大围山树蛙)

Holotype. GXNU YU000396, adult male, collected on 3 May 2021 by Guohua Yu from Dawei Mountain, Pingbian County, Yunnan Province, China (22°54'6.32"N, 103°41'33.25"E, 1740 m a.s.l.).

Paratypes. GXNU YU000392–000395, four adult females, collected on 3 May 2021 from the type locality by Guohua Yu; KIZL040278–040279, two adult males, collected on 9 March 2021 by Shuo Liu from the type locality.

Etymology. The species epithet is named for Dawei Mountain, Pingbian County, Yunnan, China, where the species was collected. We suggest the English common name "Daweishan tree frog" and the Chinese common name "Dà Weī Shān Shù wā (大围山树蛙)".

Diagnosis. The new treefrog species is assigned to *Zhangixalus* by the presence of intercalary cartilage between terminal and penultimate phalanges of digits, Y-shaped distal end of terminal phalanx, tips of digits expanded into large discs bearing circum-marginal grooves, and vomerine teeth present, dermal folds along limbs not significant, tarsal projections absent, green dorsal colouration and medium body size (Jiang et al. 2019). Phylogenetically, the new species is nested within the genus *Zhangixalus* with strong support (100% for BI and ML).

Zhangixalus daweishanensis sp. nov. can be distinguished from its congeners by a combination of the following characters: 1) body size medium (SVL 30.0–33.5 mm in males and 43.7–50.7 mm in females); 2) head wider than long; 3) upper half of iris orange and lower half yellow-brown; 4) heels not meeting when legs positioned at right angle to body, tibiotarsal articulation reaching posterior corner of eye; 5) IND/SVL 12.2%–13.0%

Table 6. Summary statistics of male specimens (mean ± standard deviation) and results of the *t*–test between the *Z. nanshanensis* sp. nov. (n = 7) and *Z. zhoukaiyae* (n = 6). The *t*–test was performed on the size–adjusted data, except SVL. * = $p < 0.05$, ** = $p < 0.01$.

Character	Mean ± SD (n = 7)	Mean ± SD (n = 6)	Levene's test		t–test	
	Z. nanshanensis	*Z. zhoukaiyae*	F	P–value	t	P–value
SVL	33.3 ± 2.57	34.0 ± 3.40	0.639	0.441	-0.387	0.706
HL	0.349 ± 0.010	0.338 ± 0.020	1.128	0.311	1.195	0.257
HW	0.368 ± 0.007	0.395 ± 0.025	7.052	0.022	-2.586	0.044*
SL	0.191 ± 0.069	0.147 ± 0.015	2.44	0.147	1.519	0.157
IND	0.105 ± 0.011	0.115 ± 0.009	0.137	0.719	-1.907	0.083
IOD	0.123 ± 0.008	0.154 ± 0.015	1.677	0.222	-4.497	0.001**
UEW	0.071 ± 0.002	0.154 ± 0.015	6.947	0.023	-12.971	0.000**
ED	0.124 ± 0.009	0.124 ± 0.012	0.477	0.504	0.003	0.998
TD	0.061 ± 0.003	0.069 ± 0.006	6.361	0.028	-2.937	0.022*
FHL	0.530 ± 0.025	0.523 ± 0.032	0.447	0.518	1.555	0.148
TL	0.441 ± 0.026	0.442 ± 0.025	0.082	0.78	-0.122	0.905
TFL	0.679 ± 0.028	0.682 ± 0.024	3.457	0.09	-0.133	0.896
FL	0.429 ± 0.024	0.437 ± 0.032	0.878	0.369	-0.547	0.595

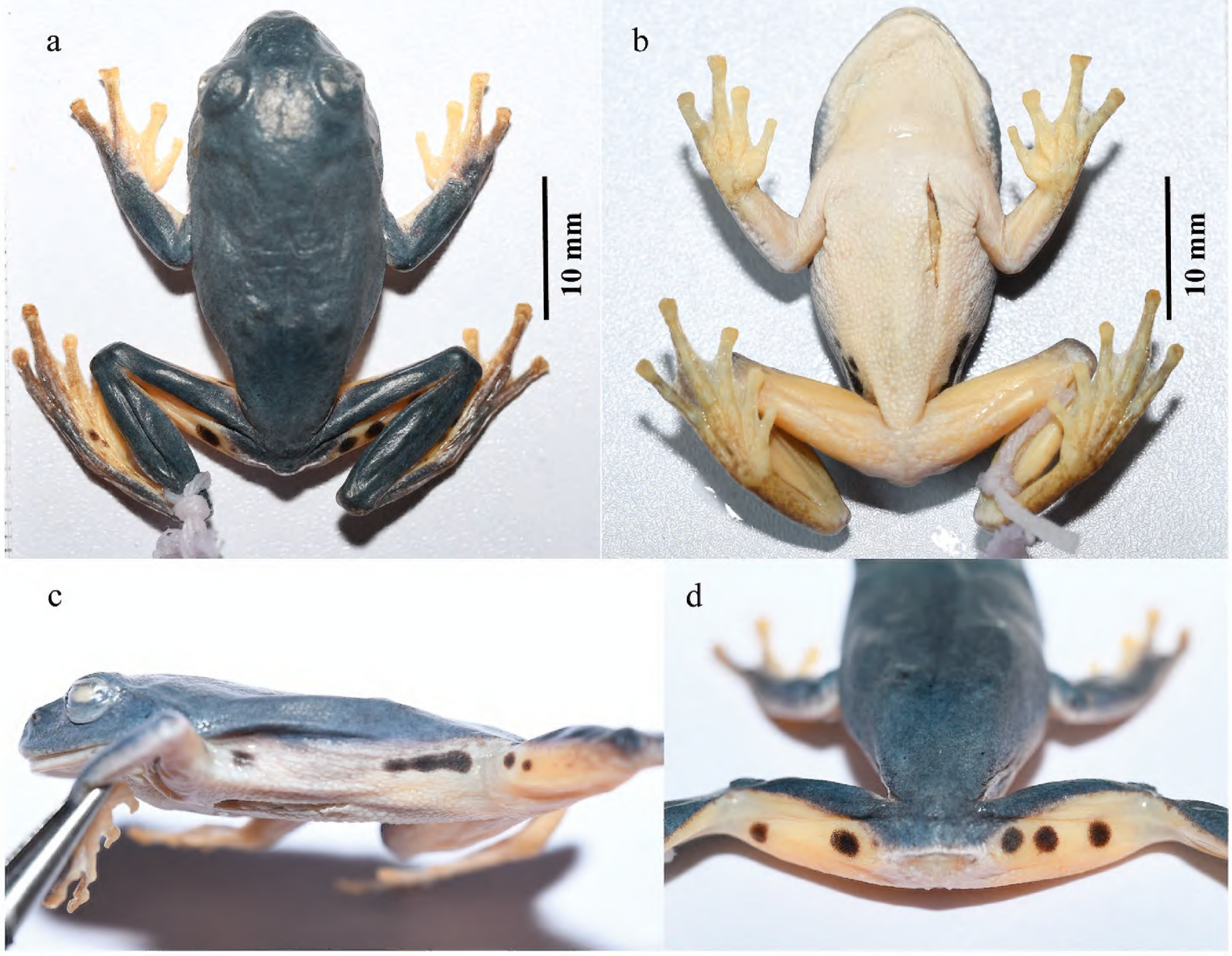

Figure 6. Dorsal (**a**), ventral (**b**), lateral (**c**), and rear (**d**) views of the holotype of *Z. daweishanensis* sp. nov. (GXNU YU000396) in preservative. Photos by Yuanqiang Pan.

in males; 6) dorsum uniformly green; 7) black blotches in axilla, groin, and posterior part of thigh; 8) vocal sac internal; 9) webbing greyish in female and yellow in male; 10) fingers webbed one third, webbing formula I2–2II1½–2⅔III2–2IV, and toes webbed half, webbing formula I1–1½II1–2III1–2½IV2½–1V; 11) throat, chest, venter, and ventral surface of limbs yolk yellow in male.

Description of holotype. Adult male, body robust, size small (SVL 33.5 mm); HL (11.3 mm) 91.9% of HW (12.3 mm); snout bluntly rounded; possesses canthus rostralis; the tip of the snout slightly protrudes beyond the edge of the lower jaw in ventral view; snout (SL 5.9 mm) longer than eye (ED 4.2 mm); nostril oval, slightly protuberant, located at the one-third between snout tip and

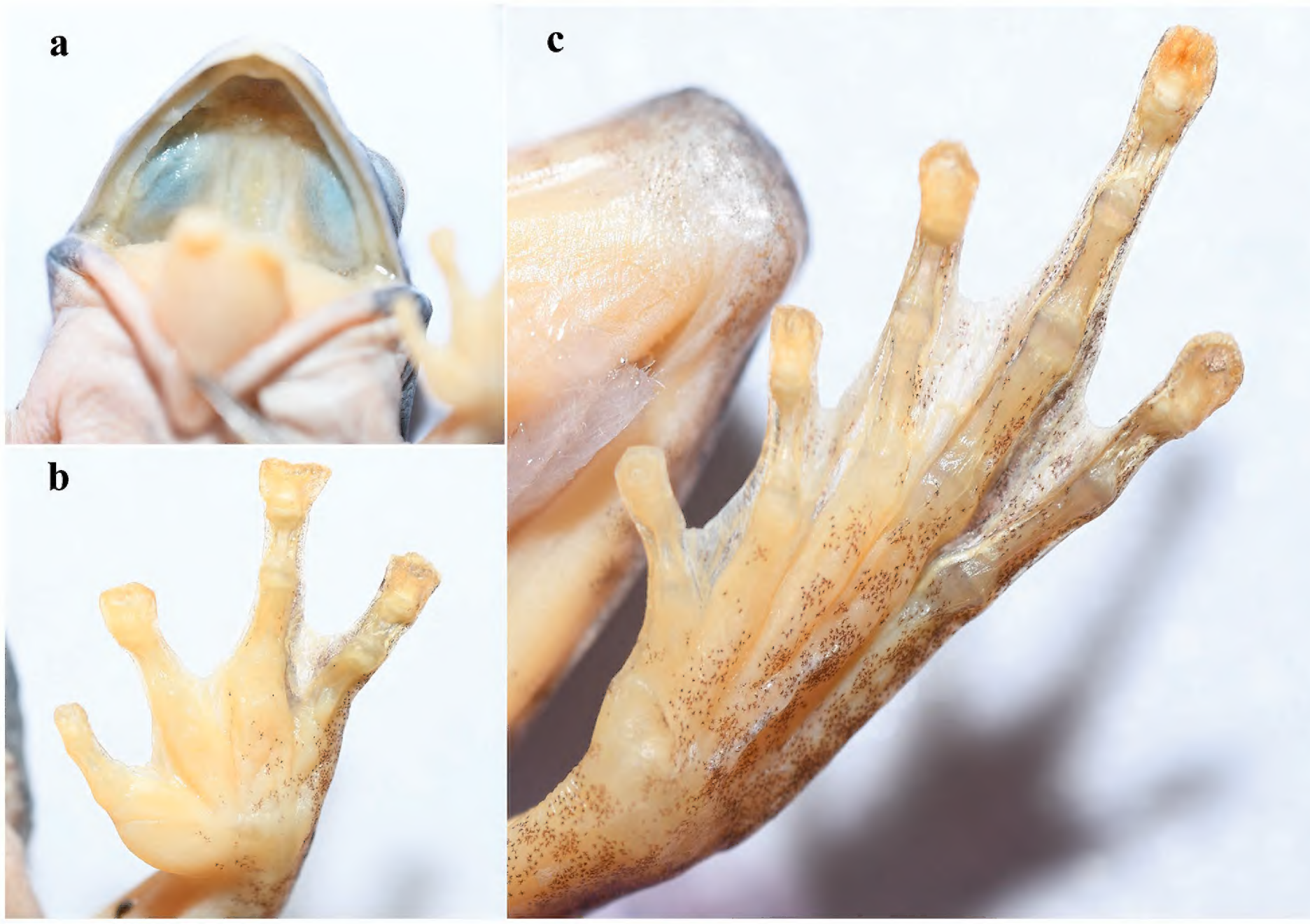

Figure 7. Views of vomerine teeth (**a**), hand (**b**), and foot (**c**) of the holotype of *Z. daweishanensis* sp. nov. (GXNU YU000396). Photos by Yuanqiang Pan.

eye; IND (4.1 mm) equal to IOD (4.3 mm) and wider than UEW (2.5 mm); pineal spot absent; pupil oval, horizontal; tympanum distinct (TD 2.0 mm), rounded, slightly smaller than half of ED (4.2 mm), nearly equal to the distance between eye and nostril (DNE 2.2 mm); supratympanic fold distinct, curves from posterior edge of eye to insertion of arm; vomerine teeth distinct; choanae oval, horizontal; tongue attached anteriorly and notched posteriorly; single internal vocal sac, with a sac slit opening on floor of mouth at each corner.

Relative length of fingers I < II < IV < III; tips of all fingers expanded into discs with circum-marginal grooves; nuptial pad present on first finger; fingers webbed one third, webbing formula I2–2II1½–2⅔III2–2IV; lateral fringe on free edge of all fingers; subarticular tubercles prominent and rounded, formula 1, 1, 2, 2; supernumerary tubercles present; inner metacarpal tubercle large, ovoid, outer metacarpal tubercle smaller in size, flattened; dermal fringe along outer edge of forearm present, not well developed.

Hind limbs moderate long, heels not meeting when legs positioned at right angle to body; tibiotarsal articulation reaching posterior margin of eye; relative length of toes I < II < V < III < IV; tibia (TL 20.6 mm) 61.5% of body size, shorter than foot (FL 21.4 mm); tips of toes expanded into discs with circum-marginal grooves, smaller than finger discs; toes webbed half, webbing formula I1–1½II1–2III1–2½IV2½–1V; subarticular tubercles prominent and rounded, formula 1, 1, 2, 3, 2; supernumerary tubercles present; inner metatarsal tubercle oval, prominent; outer metatarsal tubercle absent; dermal fringe along outer edge of tibia, tarsus, and fifth toe.

Dorsal surface of body and head smooth; dorsolateral folds absent; throat fine granular; chest, belly, and ventral surface of limbs granular.

Coloration in life. the upper half of iris orange, and the lower half yellow-brown; dorsal surface grass green; side of head and tympanic region green; lower part of flanks cream mottled with saffron yellow; throat, chest, and venter yolk yellow; limbs dorsally green and ventrally yolk yellow; anterior and posterior of thigh orange-yellow; large black blotches in axilla, groin, and posterior and anterior part of thigh; white fringes along outer edge from elbow to the fourth finger and from tibiotarsal articulation to the fifth toe; ventral surface of hand yolk yellow; webbing between fingers and toes light yellow (Fig. 8).

Coloration in preservative. Dorsal surface fades to dark blue; lower part of flanks, ventral surface of body and limbs, and posterior and anterior parts of thigh light yellow (Fig. 6).

Sexual dimorphism. The new species is sexually dimorphic, with females being distinctly larger than males and having no vocal sac and nuptial pad. Males have

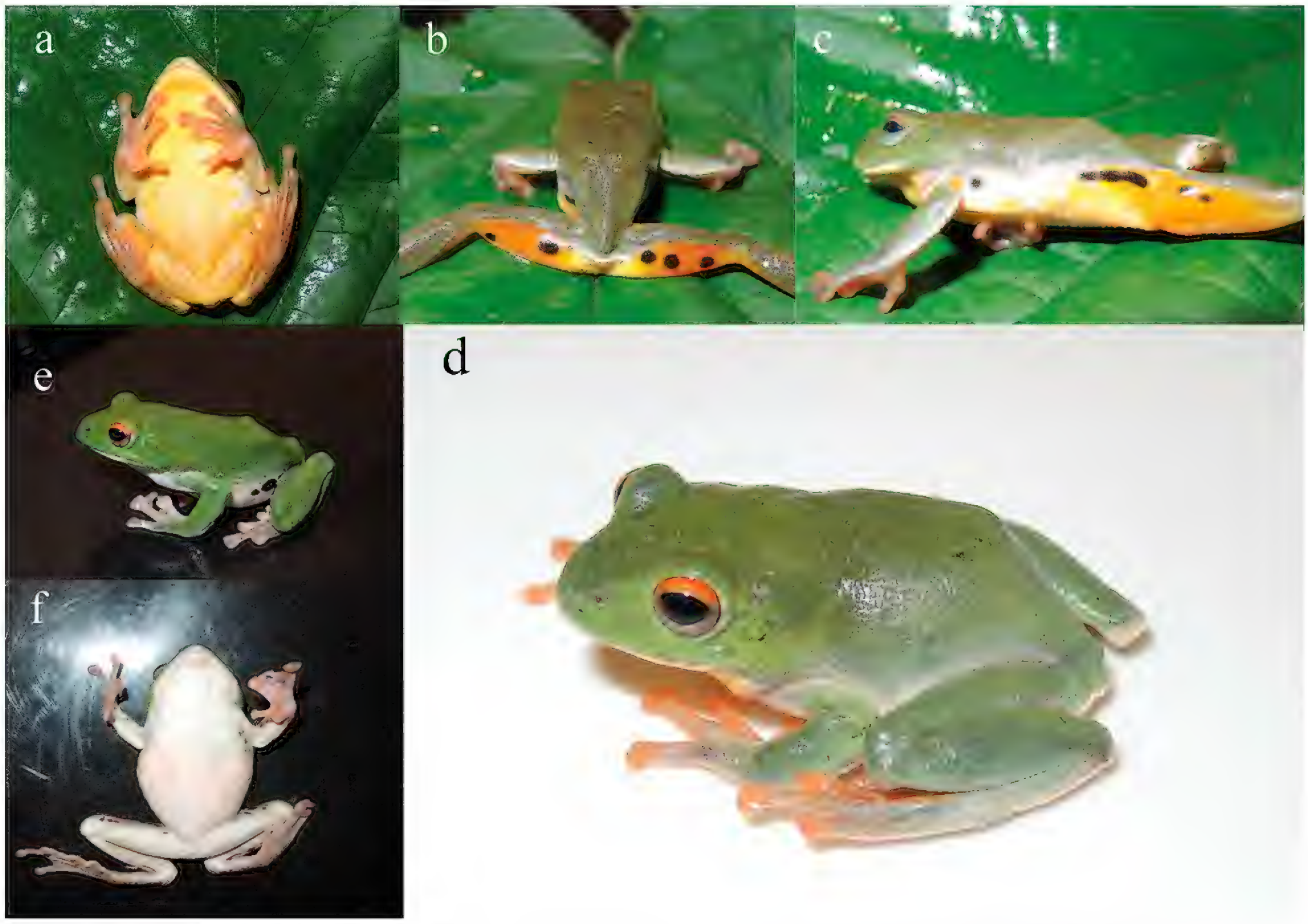

Figure 8. Views of the adult male holotype of *Z. daweishanensis* sp. nov. (GXNU YU000396; **a–d**) and adult female paratype of *Z. daweishanensis* sp. nov. (GXNU YU000393; **e, f**) in life. Photos by Guohua Yu.

internal single subgular vocal sac and orange-yellow nuptial pad on base of finger I during the breeding season. Ventral surface and anterior yolk yellow and posterior parts of thigh orange-yellow in males during breeding season.

Morphological variation. The colour pattern varied amongst individuals. Dorsal surfaces are nearly uniform green, with exceptions of paratypes GXNU YU000392 and GXNU YU000393, which have yellowish-green spots scattered on dorsal surface (Figs 8, 9). The paratypes GXNU YU000395 and KIZL040279 have no black blotch in axilla (Fig. 9). All specimens have black blotches on anterior part of thigh with the exception of the paratype KIZL040278. Moreover, number and distribution pattern of black blotches varied among specimens, as generally the black blotches in females are larger than those in males, and black blotches on thighs in males are discrete (vs. merged in females).

Distribution and ecology. The new species is currently only known from the type locality (Dawei Mountain, Pingbian County, Yunnan). During the surveys, the species was found on branches or leaves of roadside bushes near a small pond formed by a cutoff stream at the type locality (Fig. 10), and calls of this species were heard. *Feihyla palpebralis* (Smith, 1924) (Smith 1924b), *Gracixalus yunnanensis* Yu, Hui, Wang, Rao, Wu & Yang, 2019 (Yu et al. 2019b), and *Zhangixalus omeimontis* (Stejneger, 1924) were also found at the same site during the surveys.

Comparison. Phylogenetically, the new species is closely related to *Z. dorsoviridis*. *Zhangixalus daweishanensis* sp. nov. can be distinguished from *Z. dorsoviridis*, with which the new species has previously been confused, by narrower internarial distance (mean IND/SVL in males 0.126 ± 0.004 [0.122–0.130, n = 3] vs. 0.143 ± 0.007 [0.136–0.151, n = 5]), narrower tympanum (mean TD/SVL in males 0.066 ± 0.004 [0.060–0.069, n = 3] vs. 0.081 ± 0.003 [0.075–0.083, n = 5]) (Table 3), ventral, anterior, and posterior surface of thigh orange-yellow and dorsomedial surface of tibia and tarsus yolk yellow in males (vs. orange-red) (Suppl. material 4: fig. S1), webbing between fingers and toes light yellow (vs. gray or yellow-greenish) (Ostroshabov et al. 2013), nuptial pad present (vs. absent), and heels not meeting when legs positioned at right angle to body (vs. overlap) (Orlov et al. 2001).

The newly collected specimens are also morphologically similar to *Z. thaoae*, which was confused with *Z. dorsoviridis* in Vietnam. *Zhangixalus daweishanensis* sp. nov. can be distinguished from *Z. thaoae* by the upper half of the iris is orange and the lower half is yellow-brown (vs. iris red-bronze), webbing light yellow (vs. orange with some gray pattern); internal single subgular vocal sac (vs. external single subgular vocal sac), nuptial pad orange-yellow (vs. grey), heels not meeting when legs positioned at right angle to body (vs. overlapping), and ventral surface of limbs granular (vs. smooth) (Table 7).

Table 7. Morphological comparisons of *Z. daweishanensis* sp. nov., *Z. nanshanensis* sp. nov., *Z. dorsoviridis*, *Z. nigropunctatus*, *Z. lishuiensis*, *Z. thaoae*, *Z. yunnanensis*, and *Z. zhoukaiyae*.

Species	*Z. daweishanensis* sp. nov.	*Z. nanshanensis* sp. nov.	*Z. dorsoviridis*	*Z. nigropunctatus*	*Z. zhoukaiyae*	*Z. lishuiensis*	*Z. yunnanensis*	*Z. thaoae*
Color of iris	The upper half of the iris is orange, and the lower half is yellow–brown	Yellowish–brown	Orange red or The upper half of the iris is orange, and the lower half is yellow–brown	Yellowish gold	Golden–yellow	Yellow	Yellowish–brown	Red–bronze
Finger webbing	Webbed one third	Webbed one third	Webbed one third	Rudimentary	Webbed half	Webbed one third	Webbed one third	Webbed one third
Toe webbing	Webbed half	Webbed half	Webbed half	Webbed half	Webbed 2/3	Webbed half	Webbed half	Webbed half
Webbing color	Light yellow	Webbing between inner three fingers and toes light yellow and webbing between fingers III and IV and between outer three toes greyish in males	Gray or greenish yellow	Grayish	Grayish	Golden yellow	Grayish	Ventral side of webbing orange with some gray pattern
Coloration of dorsum	Green	Uniformly green or green with some irregularly indigo blue patch	Green or green with small yellow spots scattered on the dorsal surface	Green or green with small yellow spots scattered on the dorsal surface	The dorsal and lateral sides/surfaces of the body are bright green without spots	Uniformly green or green with with sparse light blue–green specks	Green or green with small yellow spots scattered on the dorsal surface	Dorsal surface of the head and body green without spots
Coloration of belly	Yolk yellow	Bright yellow	White or yellow	Grayish–white	Yellowish–white	Golden yellow	Cream white mottled with yolk yellow	Light yellow
Colorations of flanks	Cream mottled with saffron yellow	Yellow with faint small black mottled shadows	Grayish–white or orange red	Yellow	Cream bellow with small brow spots	Golden yellow	Cream mottled with greyish–brown	Cream with a black blotch
Colorations of thighs	Orange–yellow	Bright yellow	Bright orange–red	Yellow or grayish	Yellowish with grayish blotching	Golden yellow	Light yellow	Orange
Black blotches in axilla, groin and posteriorpart of thigh	Unmarked or with large and conspicuous black spots.	Unmarked or densely covered with small specks	Large and conspicuous	Large	decorated with irregular grayish blotching	Unmarked or with small light gray spots	Large	Anterior part of thigh and ventral surface of tibia orange without spots; posterior parts of thigh orange with a large black blotch
Coloration of throat	Yolk yellow	White mottled with pale yellow and faint small black spots	White or yellow	The edge of the lower lip along the throat is dark gray	Yellowish–white	White	Black	Lower jaw cream, with grey marbling, and throat region white
Vocal sac	Internal	External	Internal	External	External	Internal	External	External
Source	this study	this study	Orlov et al. 2001; Zhang et al. 2011; Ostroshabov et al. 2013	Liu et al. 1962; Fei et al. 2012	Pan et al. 2017	Liu et al. 2017	Pan et al. 2024	Nguyen et al. 2024

Compared to other species with relatively similar body size, the new species can be easily distinguished from *Z. achantharrhena* (Lue, Lai & Chen, 1994), *Z. dulitensis*, *Z. faritsalhadii* Gonggoli, Munir, Kaprawi, Kirschey & Hamidy, 2024, *Z. jarujini* (Matsui & Panha, 2006), *Z. puerensis* (He, 1999) and *Z. wui* (Li, Liu, Chen, Wu, Murphy, Zhao, Wang & Zhang, 2012) by dorsum uniformly green (vs. green with black and white spots in *Z. achantharrhena*, yellowish-green with a few purplish dots on head and back and a purplish line round snout in *Z. dulitensis*, yellowish-green in *Z. faritsalhadii*, brownish with dark marking in *Z. jarujini*, green with many reddish-brown blotches edged with dark brown in *Z. puerensis* and dark yellowish-brown to light green with

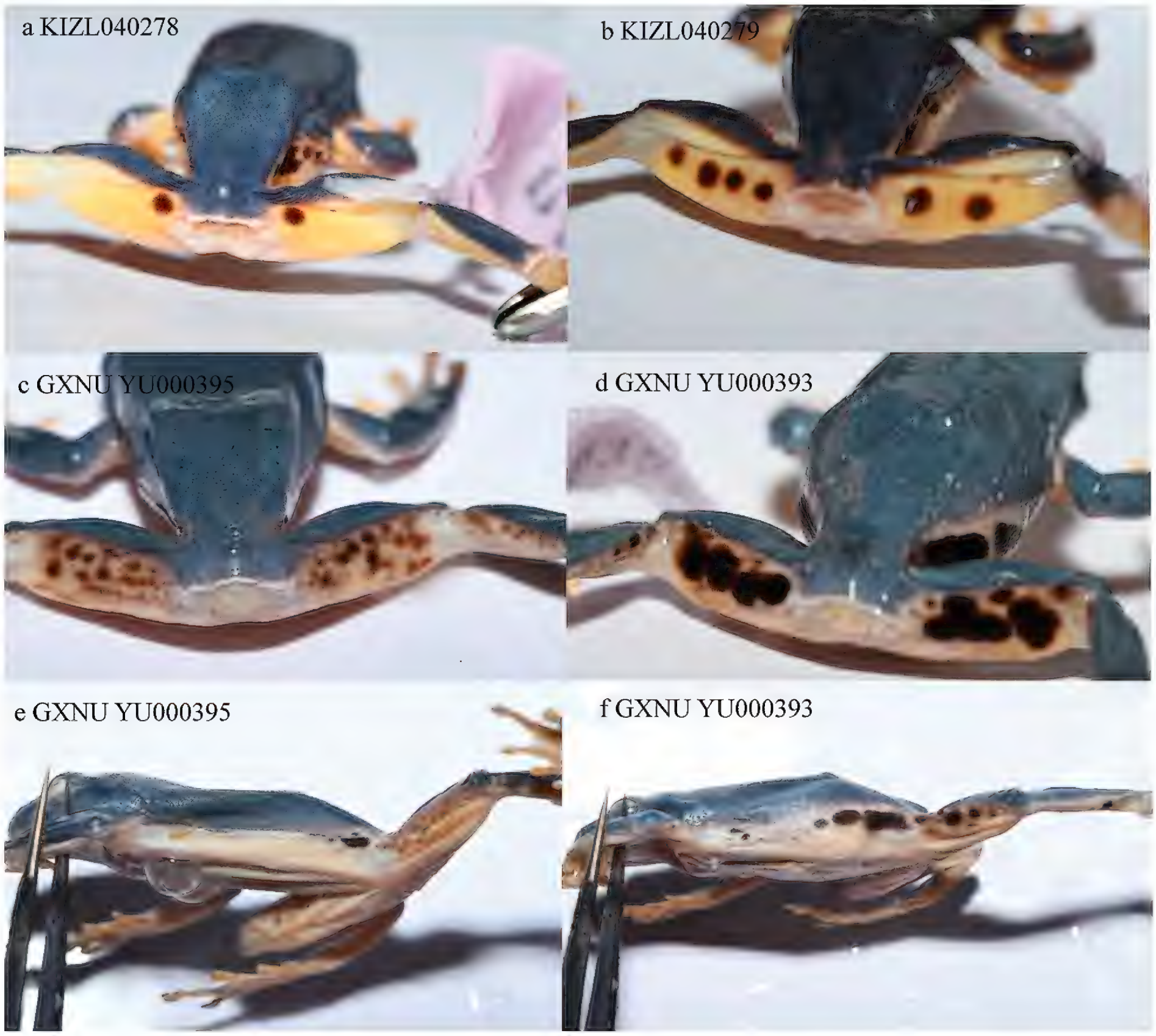

Figure 9. Morphological variation in color pattern of *Z. daweishanensis* sp. nov. in preservative. Photos by Yuanqiang Pan.

numerous light-brown spots in *Z. wui*); from *Z. chenfui* (Liu, 1945), *Z. hungfuensis* (Liu & Hu, 1961), *Z. leucofasciatus* (Liu & Hu, 1962), *Z. lishuiensis*, *Z. minimus* (Rao, Wilkinson & Liu, 2006), *Z. suffry* (Bordoloi, Bortamuli & Ohler, 2007), *Z. taipeianus* (Liang & Wang, 1978) and *Z. yaoshanensis* (Liu & Hu, 1962) by having black blotches in axilla, groin and posterior part of thigh (vs. absent); from *Z. jodiae*, *Z. melanoleucus* Brakels, Nguyen, Pawangkhanant, Idiiatullina, Lorphengsy, Suwannapoom & Poyarkov, 2023, *Z. moltrechti* (Boulenger, 1908), *Z. nigropunctatus*, *Z. pinglongensis* (Mo, Chen, Liao & Zhou, 2016), *Z. yunnanensis*, and *Z. zhoukaiyae* by vocal sac internal (vs. external); and from *Z. schlegelii* by throat yolk yellow (vs. throat faintly mottled with brown) (Suppl. material 3).

The new species is distinguishable from *Z. amamiensis* (Inger, 1947), *Z. arboreus* (Okada & Kawano, 1924), *Z. arvalis* (Lue, Lai & Chen, 1995), *Z. aurantiventris* (Lue, Lai & Chen, 1994), *Z. burmanus* (Andersson, 1939), *Z. dennysi* (Blanford, 1881), *Z. duboisi* (Ohler, Marquis, Swan & Grosjean, 2000), *Z. dugritei*, *Z. feae* (Boulenger, 1893), *Z. franki* Ninh, Nguyen, Orlov, Nguyen & Ziegler, 2020, *Z. hongchibaensis* (Li, Liu, Chen, Wu, Murphy, Zhao, Wang & Zhang, 2012), *Z. hui*, *Z. omeimontis*, *Z. owstoni* (Stejneger, 1907), *Z. pachyproctus* Yu, Hui, Hou, Wu, Rao & Yang, 2019, *Z. prasinatus* (Mou, Risch & Lue, 1983), *Z. prominanus* (Smith, 1924), *Z. smaragdinus* (Blyth, 1852), *Z. viridis* (Hallowell, 1861), and *Z. yinggelingensis* (Chou, Lau & Chan, 2007) by smaller body size (SVL 32.0–33.5 mm) (vs. SVL > 50 mm in the following species) and a combination of the following characters: dorsal surface uniformly green and no brown stripe along canthus rostralis and supratympanic fold (vs. dorsal surface green with brown blotches and a brown stripe along canthus rostralis in *Z. burmanus*, *Z. duboisi*, *Z. hongchibaensis*, *Z. hui*, and *Z. omeimontis*, green or dark brown with irregularly brown spots in *Z. dugritei*); the upper half of iris orange and the lower half yellow-brown (vs. greenish in *Z. amamiensis*, *Z. burmanus*, *Z. arboreus*, reddish in

Figure 10. Habitat of *Z. daweishanensis* sp. nov. at the type locality. Photo by Guohua Yu.

Z. prominanus, yellow in *Z. arvalis*, pale yellow in *Z. aurantiventris*, yellowish-gold in *Z. dennysi*, *Z. omeimontis*, *Z. prasinatus*, and *Z. smaragdinus*, dark gold in *Z. duboisi*, yellowish-brown in *Z. dugritei*, *Z. hongchibaensis*, reddish brown in *Z. hui*, yellowish gold mottled with silver in *Z. owstoni*, silver in *Z. yinggelingensis*); internal vocal sac (vs. external vocal sac in *Z. arvalis*, *Z. dugritei*, *Z. hui*, and *Z. prasinatus*); and black blotches in axilla, groin and anterior and posterior part of thighs (vs. absent *Z. arvalis*, *Z. aurantiventris*, *Z. dennysi*, *Z. feae*, *Z. franki*, *Z. pachyproctus*, *Z. prominanus* and *Z. smaragdinus*) (Suppl. material 3).

Zhangixalus nanshanensis sp. nov.

https://zoobank.org/68E0B4AE-3831-43BA-B5AD-EB5D5B39DBE8

Figs 11–13

Nanshan tree frog/Nān Shān Shù wā (南山树蛙)

Holotype. GXNU YU000771, adult male, collected on 5 April 2024 by Guohua Yu, Shangjing Tang, and Ju Chen from Nanshan National Park, Chengbu County, Hunan Province, China (26°8'50.17"N, 110°8'4.31"E, 1785 m a.s.l.).

Paratypes. Six adult males (GXNU YU000772–YU000776, GXNU YU000778) and an adult female (GXNU YU000777) were collected on 5 April 2024 by Guohua Yu, Shangjing Tang, and Ju Chen from the type locality.

Etymology. The species epithet is named for Nanshan National Park, Chengbu County, Hunan, China, where the species was collected. We suggest the English common name "Nanshan tree frog" and the Chinese common name "Nān Shān Shù wā (南山树蛙)".

Diagnosis. The new treefrog species is assigned to *Zhangixalus* by the presence of intercalary cartilage between terminal and penultimate phalanges of digits, Y-shaped distal end of terminal phalanx, tips of digits expanded into large discs bearing circum-marginal grooves, and vomerine teeth present, dermal folds along limbs not significant, tarsal projections absent, green dorsal colouration and medium body size (Jiang et al. 2019). Phylogenetically, the new species is nested within the genus *Zhangixalus* with strong support (100% for BI and 99% for ML).

Zhangixalus nanshanensis sp. nov. can be distinguished from its congeners by a combination of the following characters: 1) body size medium (SVL 28.1–36.7 mm in males); 2) head width less wider than long; 3) iris yellowish-brown; 4) heels not meeting when legs positioned at right angle to body, tibiotarsal articulation reaching posterior corner of eye; 5) dorsum uniformly green or green with some irregularly indigo blue patch; 6) posterior part of thigh yellow with small black blotches; 7) ventral side of hand and foot bright yellow with irregular gray-brown spots; 8) flanks cream mottled with saffron yellow and have faint small black mottled shadows; 9) vocal sac external; 10) throat pale saffron yellow with faint small black spots; 11) webbing between fingers and toes greyish in females, and webbing between inner three fingers and toes light yellow, and webbing between fingers III and IV and between outer three toes greyish in males; 12) fingers webbed one third, webbing formula I2–2⅓II1½–3III2⅓–2IV, and toes webbed half, webbing formula I1–2II1–2III1–2½IV3–1V.

Description of holotype. Adult male, body robust, size small (SVL 33.7 mm); head length slightly less than the head width, HL (12.0 mm) 99.2% of HW (12.1 mm); snout bluntly rounded, possesses canthus rostralis, the tip of the snout slightly protrudes beyond the edge of the lower jaw in ventral view; snout (SL 5.2 mm) longer than eye (ED 4.6 mm); nostril oval, slightly protuberant, located at the one-third between snout tip and eye; IND (3.9 mm) shorter than IOD (4.2 mm) and wider than UEW (2.4 mm); pineal spot absent; pupil oval, horizontal; tympanum small (TD 2.1 mm), rounded, smaller than ED (4.6 mm), nearly equal to the distance between eye and nostril (DNE 2.2 mm); supratympanic fold distinct, curves from posterior edge of eye to insertion of arm; vomerine teeth distinct; choanae oval, horizontal; tongue attached anteriorly and notched posteriorly; single external vocal sac, with a sac slit opening on floor of mouth at each corner.

Relative length of fingers I < II < IV < III; tips of all fingers expanded into discs with circum-marginal grooves; nuptial pad present on first finger; fingers webbed one third, webbing formula I2–2⅓II1½–3III2⅓–2IV; lateral fringe on free edge of all fingers; subarticular tubercles prominent and rounded, formula 1, 1, 2, 2; supernumerary tubercles present; inner metacarpal tubercle large, ovoid, outer metacarpal tubercle small, flattened, and divided into two; dermal fringe along outer edge of forearm present, not well developed.

Hind limbs moderate long, heels not meeting when legs positioned at right angle to body; tibiotarsal articulation reaching posterior margin of eye; relative length of toes I < II < V < III < IV; tibia (TL 15.0 mm) 44.5% of body size, longer than foot (FL 14.6 mm); tips of toes expanded into discs with circum-marginal grooves, smaller than finger discs; toes webbed half, webbing formula I1–2II1–2III1–2½IV3–1V; subarticular tubercles prominent and rounded, formula 1, 1, 2, 3, 2; supernumerary tubercles present; inner metatarsal tubercle oval, prominent; outer metatarsal tubercle absent.

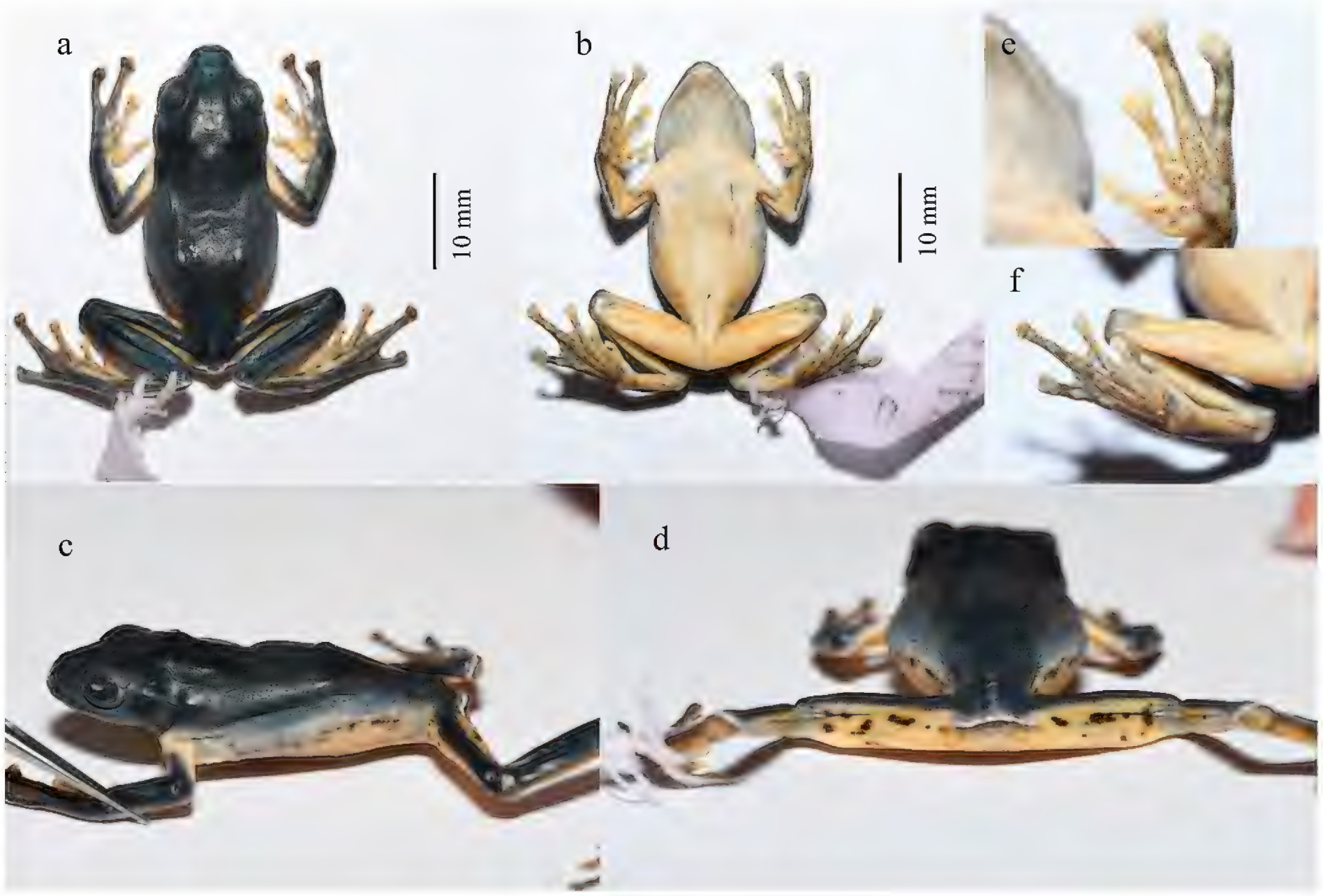

Figure 11. Views of the adult male holotype of *Z. nanshanensis* sp. nov. (GXNU YU000771) in preservative. Photos by Yuanqiang Pan.

Dorsal surface of body and head smooth; dorsolateral folds absent; throat granular; chest, belly, and ventral surface of limbs granular.

Coloration in life. Iris yellowish-brown; dorsal surface grass green; side of head and tympanic region green; body of flanks green mottled with yellow; throat white mottled with pale yellow and faint small black spots; chest, abdomen, ventral surface of limbs, and dorsomedial surface of tibia and tarsus bright yellow; anterior and posterior parts of thigh yellow with small black blotches; faint small black mottled shadows on flank and groin; white fringes along outer edge from tibiotarsal articulation to the fifth toe; ventral surface of hand and foot yellow with greyish-brown spots; webbing between inner three fingers and toes light yellow and webbing between fingers III and IV and between outer three toes greyish; dorsal side of discs of fingers I and II and discs of toes I–III light yellow and dorsal side of discs of fingers III and IV and dorsal side of discs of toes IV and V greyish-brown (Fig. 12).

Coloration in preservative. Dorsal surface dark blue; lower part of flanks and ventral surface of body and limbs cream, throat milky white covered with greyish pattern; ventral surface of hand and foot cream with irregular gray-brown spots.

Sexual dimorphism. The new species is sexually dimorphic, with females being distinctly larger than males and having no vocal sac and nuptial pad. Males have external single subgular vocal sac and light-yellow nuptial pad on base of finger I. Ventral surface and anterior and posterior parts of thigh yolk yellow in males (vs. cream white in females) during breeding season.

Morphological variation. The colour pattern varied amongst individuals. The paratype GXNU YU000772 has only a few irregularly black spots on posterior surface of thigh, the paratype GXNU YU000775 has no large black spots on the posterior surface of thigh, and these two paratypes have no large black blotches on flank (Fig. 13). Throat of the female paratype GXNU YU000777 is white mottled with a few irregular dark blotches (Fig. 12). The paratypes GXNU YU000777 and GXNU YU000773 have some irregularly indigo blue patches on dorsum (Fig. 12). In addition, coloration of the new species in life can vary as day period and microhabitat change; dorsum is lighter nocturnally than during the day, with dorsal surfaces appearing light to dark green and even dark brown.

Distribution and ecology. The new species is currently known from the type locality (Nanshan National Park, Chengbu County, Hunan, China). During the surveys, the species was found calling in wet meadows nearby a pond at the type locality (Fig. 14). *Zhangixalus minimus* was also found at the same site during the surveys.

Comparison. Phylogenetically, the new species is closely related to *Z. lishuiensis* and *Z. zhoukaiyae*. *Zhangixalus nanshanensis* sp. nov. can be distinguished from *Z. lishuiensis* by vocal sac external (vs. internal), throat light yellow mottled with black spots in males (vs. white), shorter head (Fig. 15), outer metacarpal tubercle present (vs. absent), and

Figure 12. Views of the adult male holotype of *Z. nanshanensis* sp. nov. (GXNU YU000771; **a, b**) and adult female paratype of *Z. nanshanensis* sp. nov. (GXNU YU000777; **c, d**) in life. Photos by Guohua Yu

flanks cream mottled with saffron yellow and having faint small black mottled shadows (vs. flanks yellow). *Zhangixalus nanshanensis* sp. nov. can be distinguished from *Z. zhoukaiyae* by throat yellow with greyish brown blotches (vs. pure paler yellowish without black), outer metatarsal tubercle absent (vs. present), upper eyelid width smaller than interorbital distance (vs. upper eyelid width approximately equal to interorbital distance), and outer fingers webbed one third and outer toes webbed half (vs. fingers webbed half and outer toes webbed two thirds) (Tables 6, 7).

Zhangixalus nanshanensis sp. nov. can be distinguished from *Z. nigropunctatus*, with which the new species has previously been confused, by fingers webbed one third (vs. finger web rudimentary or 1/4 webbed), tibiotarsal articulation reaching posterior margin of eye (vs. reaching posterior margin of tympanum), ventral surface yellow in males (vs. greyish white), outer metatarsal tubercle absent (vs. present), snout steeper in profile (Fig. 15), and wider head, narrower upper eyelid, smaller tympanum, longer tibia, and longer foot and tarsus (Suppl. material 4: table S4).

Zhangixalus nanshanensis sp. nov. can be distinguished from *Z. yunnanensis* by throat light yellow mottled with greyish (vs. throat dark black), snout steeper in profile (Fig. 15), and fingers webbed nearly half (vs. rudimentary) (Table 7).

Zhangixalus nanshanensis sp. nov. can be distinguished from *Z. dorsoviridis* by iris yellowish-brown (vs. the upper half of iris orange and the lower half yellow-brown), external single subgular vocal sac (vs. internal single subgular vocal sac), throat yellow mottled with faint small black spots (vs. lacking dark spots on throat), ventral, anterior, and posterior surface of thigh and dorsomedial surface of tibia and tarsus yolk yellow in males (vs. orange-red; Suppl. material 4: fig. S1), and heels not meeting when legs positioned at right angle to body (vs. overlap) (Table 7).

Zhangixalus nanshanensis sp. nov. can be distinguished from *Z. thaoae* by iris yellowish-brown (vs. red-bronze), webbing between inner three fingers and toes light yellow, and webbing between fingers III and IV and between outer three toes greyish in males (vs. orange

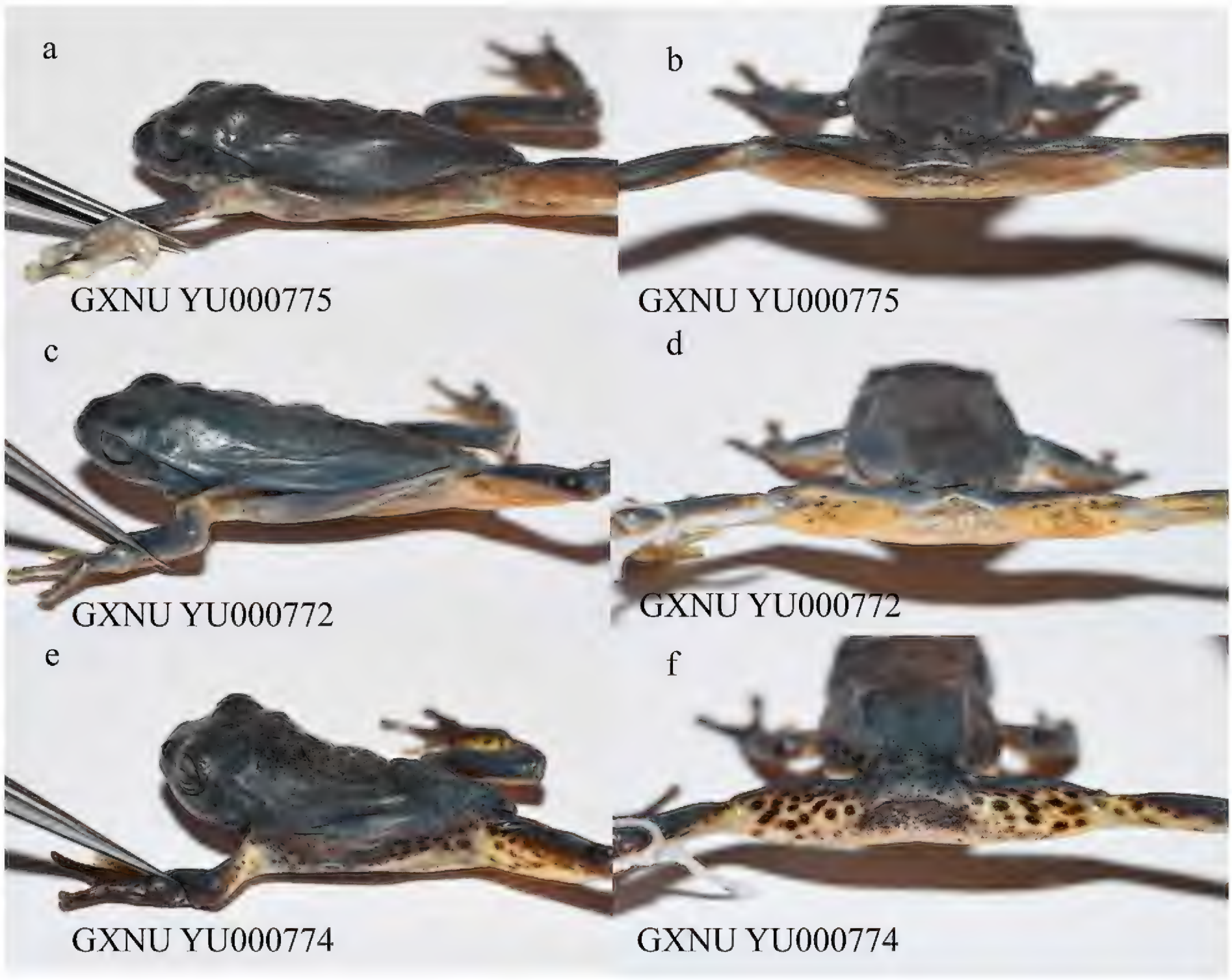

Figure 13. Morphological variation in color pattern of *Z. nanshanensis* sp. nov. in preservative. Photos by Yuanqiang Pan.

with some gray pattern), and heels not meeting when legs positioned at right angle to body (vs. overlap) (Table 7).

Zhangixalus nanshanensis sp. nov. can be distinguished from *Z. daweishanensis* sp. nov. by iris yellowish-brown (vs. the upper half of iris orange and the lower half yellow-brown; Suppl. material 4: fig. S1), external single subgular vocal sac (vs. internal single subgular vocal sac), throat yellow mottled with faint small black spots in males (vs. yellow with no black spots), and webbing between outer two fingers and webbing between outer three toes greyish in males (vs. yellow) (Table 7).

In body size, besides *Z. dorsoviridis*, *Z. lishuiensis*, *Z. nigropunctatus*, *Z. daweishanensis* sp. nov., *Z. thaoae*, *Z. yunnanensis*, and *Z. zhoukaiyae*, the new species is relatively similar to *Z. achantharrhena*, *Z. chenfui*, *Z. dulitensis*, *Z. hungfuensis*, *Z. faritsalhadii*, *Z. jarujini*, *Z. jodiae*, *Z. leucofasciatus*, *Z. melanoleucus*, *Z. minimus*, *Z. moltrechti*, *Z. pinglongensis*, *Z. puerensis*, *Z. schlegelii*, *Z. suffry*, *Z. taipeianus*, *Z. wui*, and *Z. yaoshanensis*. The new species can be easily distinguished from *Z. achantharrhena*, *Z. dulitensis*, *Z. faritsalhadii*, *Z. jarujini*, *Z. puerensis* and *Z. wui* by dorsum uniformly green (vs. green with black and white spots in *Z. achantharrhena*, yellowish-green with a few purplish dots on head and

Figure 14. Habitat of the *Z. nanshanensis* sp. nov. at the type locality. Photo by Guohua Yu

dorsum and a purplish line round snout in *Z. dulitensis*, yellowish-green with numerous dark spot and irregular blotches cream in *Z. faritsalhadii*, brownish with dark marking in *Z. jarujini*, green with many reddish-brown blotches edged with dark brown in *Z. puerensis* and dark yellowish-brown to light green with numerous light-brown spots in *Z. wui*); from *Z. chenfui*, *Z. hungfuensis*, *Z. leucofasciatus*, *Z. minimus*, *Z. suffry*, *Z. taipeianus* and

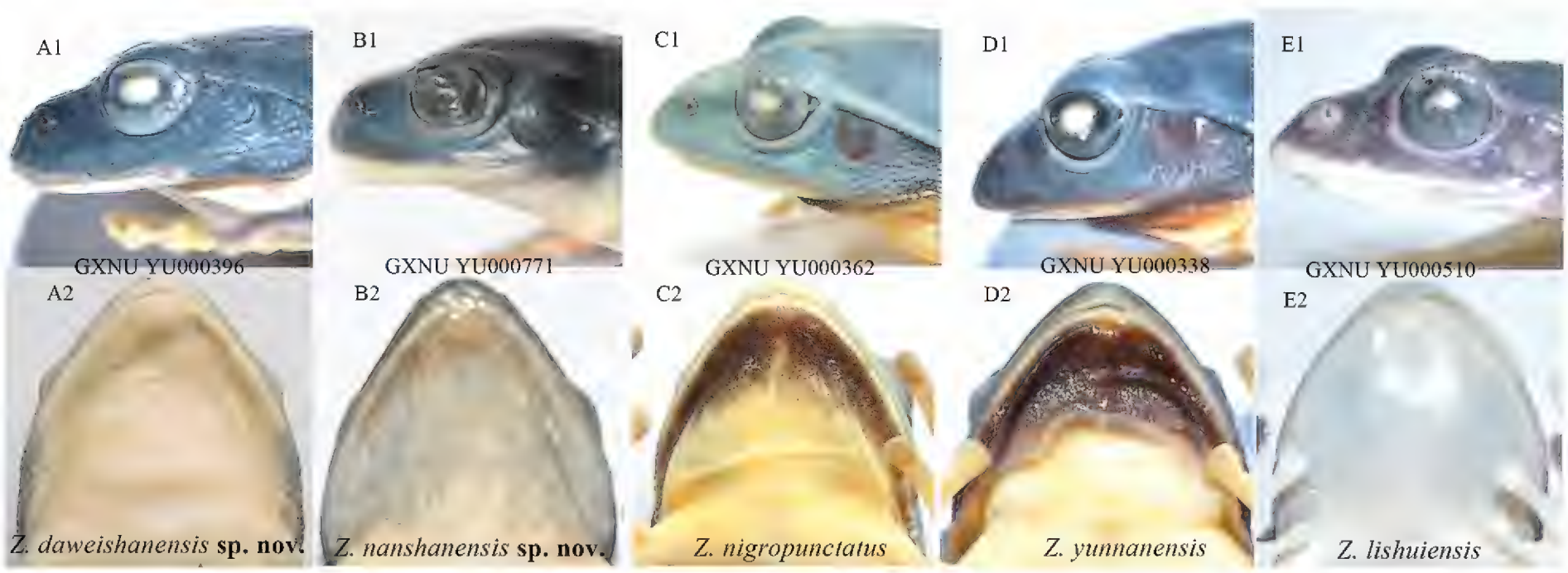

Figure 15. Lateral views of the head and throat of *Z. daweishanensis* sp. nov., *Z. nanshanensis* sp. nov., *Z. nigropunctatus*, *Z. yunnanensis*, and *Z. lishuiensis*. Photo by Guohua Yu.

Z. yaoshanensis by having black blotches in axilla, groin and posterior part of thigh (vs. absent); from *Z. schlegelii* by vocal sac external (vs. internal); from *Z. jodiae*, *Z. moltrechti*, and *Z. pinglongensis* by throat saffron yellow mottled with faint small black spots (vs. greyish in *Z. jodiae*, white in *Z. moltrechti*, and white with slightly gray background in *Z. pinglongensis*); and from *Z. melanoleucus* by webbing between inner three fingers and toes light yellow and webbing between fingers III and IV and between outer three toes greyish (vs. mottled with black blotches in *Z. melanoleucus*) (Suppl. material 3).

The new species is distinguishable from *Z. amamiensis*, *Z. arboreus*, *Z. arvalis*, *Z. aurantiventris*, *Z. burmanus*, *Z. dennysi*, *Z. duboisi*, *Z. dugritei*, *Z. feae*, *Z. franki*, *Z. hongchibaensis*, *Z. hui*, *Z. omeimontis*, *Z. owstoni*, *Z. pachyproctus*, *Z. prasinatus*, *Z. prominanus*, *Z. smaragdinus*, *Z. viridis*, and *Z. yinggelingensis* by smaller body size and a combination of the following characters: 1) dorsal surface uniformly green and no brown stripe along canthus rostralis and supratympanic fold (vs. dorsal surface green with brown blotches and a brown stripe along canthus rostralis in *Z. burmanus*, *Z. duboisi*, *Z. hongchibaensis*, *Z. hui*, and *Z. omeimontis*, green or dark brown with irregularly brown spots in *Z. dugritei*); 2) iris is yellowish-brown (vs. greenish in *Z. amamiensis*, *Z. burmanus* and *Z. arboreus*, reddish in *Z. prominanus*, yellow in *Z. arvalis*, pale yellow in *Z. aurantiventris*, yellowish-gold in *Z. dennysi*, *Z. omeimontis*, *Z. prasinatus*, and *Z. smaragdinus*, dark gold in *Z. duboisi*, reddish brown in *Z. hui*, yellowish gold mottled with silver in *Z. owstoni*, and silver in *Z. yinggelingensis*); 3) external vocal sac (vs. internal vocal sac in *Z. amamiensis*, *Z. arboreus*, *Z. aurantiventris*, *Z. burmanus*, *Z. dennysi*, *Z. feae*, *Z. hongchibaensis*, *Z. omeimontis*, *Z. owstoni*, *Z. pachyproctus*, *Z. smaragdinus*, *Z. viridis* and *Z. yinggelingensis*; and 4) black blotches in axilla, groin and anterior and posterior part of thighs (vs. absent in *Z. arvalis*, *Z. aurantiventris*, *Z. dennysi*, *Z. feae*, *Z. franki*, *Z. pachyproctus*, *Z. prominanus* and *Z. smaragdinus*) (Suppl. material 3).

Discussion

It has been revealed that *Z. nigropunctatus*, which was once recorded widely in central and south-western China (Fei et al. 2012) and north-western Vietnam (Poyarkov et al. 2021), actually involves multiple misidentified populations (Yu et al. 2019a; Li et al. 2012a; Mo et al. 2016; Pan et al. 2017; Pan et al. 2024; Nguyen et al. 2024), some of which were moved into other known species (e.g., Li et al. 2012a) and some of which were described as new species (e.g., Pan et al. 2017; Pan et al. 2024; Nguyen et al. 2024). In Hunan, China, *Z. nigropunctatus* was recorded from Chengbu and Sangzhi Counties (Fei et al. 2012; AmphibiaChina 2024). In this study, based on molecular and morphological evidence, we revealed that the Chengbu population in southwestern Hunan represents an independent species of *Zhangixalus*. This result further improves our understanding of the taxonomy of the *Z. nigropunctatus* complex and implies that the taxonomy of the Sangzhi population of *Z. nigropunctatus* also needs further investigation. In addition, *Z. nigropunctatus* is also recorded from southeastern Guizhou (Leishan County; AmphibiaChina 2024), which is adjacent to southwestern Hunan. It is possible that the Leishan population belongs to *Z. nanshanensis* sp. nov. pending additional data. Similarly, it could be expected that this new species would be found in adjacent northeastern Guangxi (Fig. 1).

It is a little surprise that there is a novel lineage of *Zhangixalus* (*Z. daweishanensis* sp. nov.) allied to *Z. dorsoviridis* at Mt. Dawei, Pingbian, Yunnan. Yu et al. (2009) and Li et al. (2012a) found that *Z. nigropunctatus* from Pingbian and Jinping did not group together with topotypes of this species, and Li et al. (2012a) placed these two populations into *Z. dorsoviridis* as they are closely related to *Z. dorsoviridis* with low genetic divergence. Around the same time, Zhang et al. (2011) reported the first record of *Z. dorsoviridis* from China based on samples collected from Mt. Dawei, Pingbian. In this study, we found the newly collected specimens from Mt. Dawei, Pingbian, form a distinct clade, being sister to *Z. dorsoviridis*,

and morphologically can be distinguished from *Z. dorsoviridis* by a series of characters. It is interesting that there are two sibling species living in sympatry at Mt. Dawei, Pingbian, and additional studies are required to investigate the mechanism of speciation of these two species.

In addition, more studies are required to unravel the taxonomy and distribution of *Z. dorsoviridis*, which is exactly known from northern Vietnam (Sa Pa) and southern Yunnan (Jinping and Pingbian) based on the present and previous phylogenetic analyses (Li et al. 2012a; Pan et al. 2024). Hou et al. (2017) recorded this species from Maoershan National Nature Reserve, Guilin, Guangxi. Geographically, this site is far away from northern Vietnam and southern Yunnan, and morphologically, the Maoershan population differs from *Z. dorsoviridis* from northern Vietnam and southern Yunnan in color pattern (Fig. 16) and nuptial pad, which is absent in *Z. dorsoviridis* according to Orlov et al. (2001) but present on the base of finger I in the Maoershan population (Hou et al. 2017). Therefore, we consider that the taxonomy of nominal *Z. dorsoviridis* from Maoershan National Nature Reserve remains unclear. Additionally, the specimen VNMN 4099, which was collected from Son La, Vietnam, and was identified as *Z. dorsoviridis* (Nguyen et al. 2014), was recovered as sister to *Z. thaoae*, indicating that the taxonomic status of this specimen remains undetermined and probably represents a cryptic species. Being inconsistent with the phylogenetic analyses, the ASAP analysis assigned *Z. jodiae* (voucher number: VNMN 07122) and VNMN 4099 into one taxon, and the genetic distance between them was estimated to be zero. This is due to the fact that the sequence of VNMN 4099 (LC010577, 461 bp) overlapped only partially with the sequence of *Z. jodiae* (LC545595, 725 bp), and the overlapping region has no mutation. Furthermore, we found that the male specimen TBUPAE525 of nominal *Z. dorsoviridis* from near Nong Vai Village, Son La Province, Vietnam (Pham et al. 2017) is distinguishable from *Z. dorsoviridis* by iris yellow (vs. upper of iris bright orange; Fig. 16) and larger body size (54.1 mm vs. maximum length 43.2 mm for adult male of *Z. dorsoviridis* from the type locality), implying that this specimen from Son La Province, Vietnam, may also be a misidentification.

Recently, Dufresnes and Litvinchuk (2022) considered *Z. hui* as a synonym of *Z. dugritei*, noted that *Z. lishuiensis* is likely conspecific with *Z. zhoukaiyae*, and *Z. duboisi* is likely conspecific with *Z. omeimontis* (Dufresnes and Litvinchuk 2022). However, we consider that the taxonomic rearrangements of Dufresnes and Litvinchuk (2022) should be treated with caution. Dufresnes and Litvinchuk (2022) estimated the divergence between *Z. duboisi* and *Z. omeimontis* to be 1.5% using a sample of nominal *Z. duboisi* from Pingbian, Yunnan (voucher YN080484, GenBank accession: JX219418). However, *Z. duboisi* from Pingbian actually belongs to *Z. omeimontis* (Shui et al. 2023) and we recovered that *Z. duboisi* is sister to *Z. puerensis* and *Z. omeimontis* is sister to *Z. franki* (Fig. 2). The divergence between *Z. duboisi* and *Z. omeimontis* in 16S rRNA is estimated to be 5.1%. These results support that *Z. duboisi* and *Z. omeimontis* are distinctive from each other.

Owing to the fact that *Z. zhoukaiyae* and *Z. lishuiensis* were morphologically similar and the genetic divergence between them is relatively low, the taxonomic status of

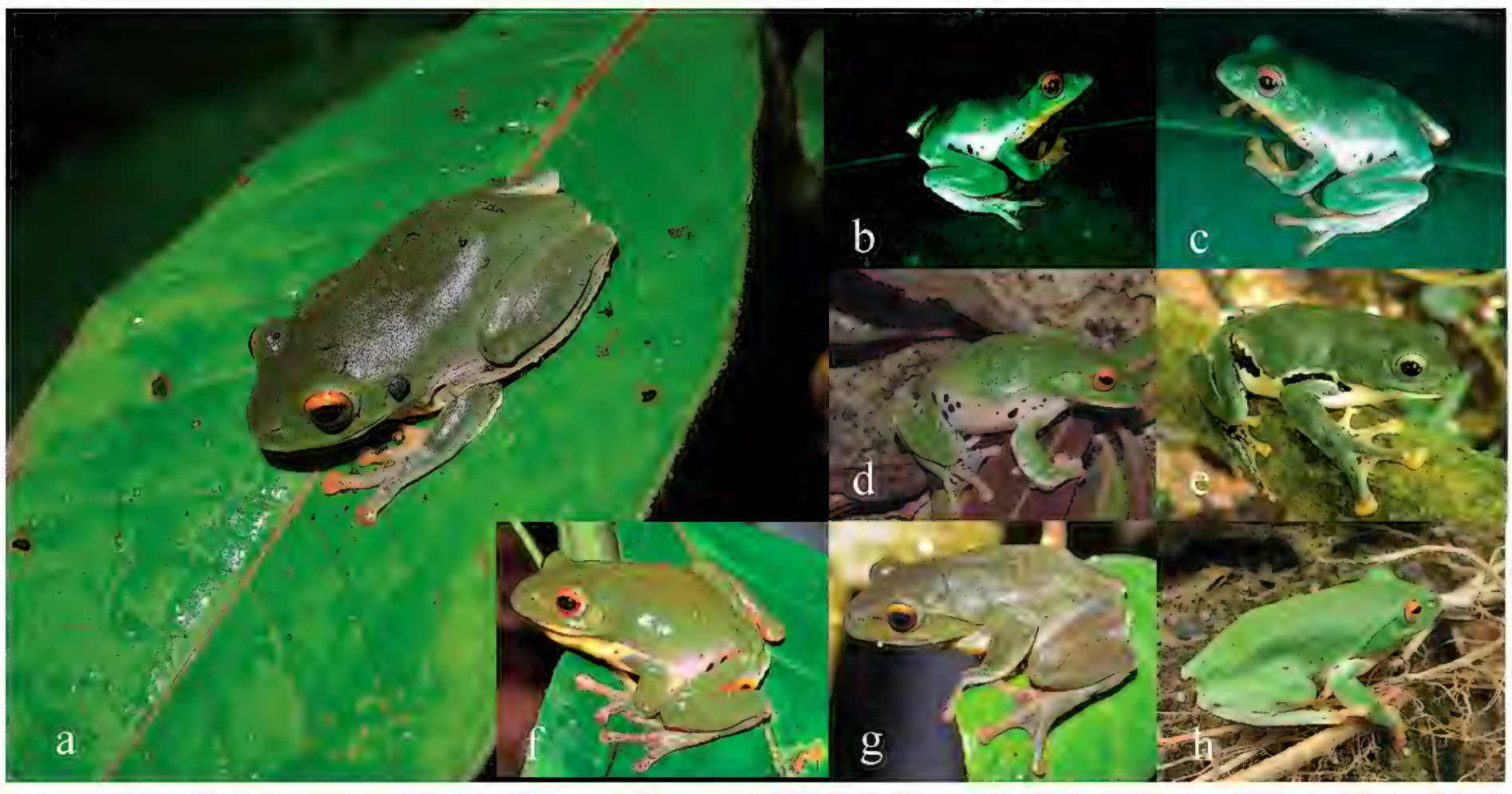

Figure 16. Views of *Z. daweishanensis* sp. nov. from the type locality (**a**; photo by Guohua Yu) and *Z. dorsoviridis* from Sa Pa, Vietnam (type locality; **b–d**), Copia NR, Vietnam (**e**; TBU PAE 525), Pingbian, Yunnan, China (**f, h**), and Mao'er Mountain, Guangxi, China (**g**). The images in **b–h** were reproduced from Orlov et al. (2001), Orlov et al. (2008), Mo et al. (2016), Pham et al. (2017), Zhang et al. (2011), Hou et al. (2017), and Pan et al. (2017), respectively.

these two species was questioned (Dufresnes and Litvinchuk 2022; Brakels et al. 2023; Hong et al. 2024). In this study, we revealed that *Z. lishuiensis* can be distinguished from *Z. zhoukaiyae* by a longer head (Fig. 5), and they were assigned into different taxa by both analyses of species delimitation. Therefore, combining the morphological distinctions outlined in the original descriptions, e.g., *Z. zhoukaiyae* has an external vocal sac (Pan et al. 2017), whereas *Z. lishuiensis* has an internal vocal sac (Liu et al. 2017) (Table 7 and Suppl. material 4: table S6), we agree with Pan et al. (2024) that *Z. zhoukaiyae* and *Z. lishuiensis* are two separate species. Additionally, although the genetic divergence between *Z. hui* and *Z. dugritei* is low, they differ from each other in karyotype structure and snout color (yellow-brown in *Z. hui* and green in *Z. dugritei*; Li et al. 2012b). So, we temporarily regard the *Z. hui* and *Z. dugritei* as two separate species, as other genetic factors (gene introgression or incomplete lineage sorting) may have led to their extremely low genetic distance.

In summary, we revealed that the species diversity in *Zhangixalus* was underestimated and described two cryptic species of this genus. Moreover, our results indicated the *Z. nigropunctatus* and *Z. dorsoviridis* complexes involve other cryptic species and misidentifications. Therefore, additional studies are required to resolve these taxonomic confusions and clarify the species diversity of the genus *Zhangixalus* based on multiple lines of evidence.

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References

AmphibiaChina (2024) The database of Chinese amphibians. Kunming Institute of Zoology (CAS), Kunming, Yunnan, China. https://www.Amphibiachina.org [Accessed 4 May 2024]

Andersson LG (1939) [1938] Batrachians from Burma collected by Dr. R. Malaise, and from Bolivia and Ecuador collected by Dr. C. Hammarlund. Arkiv för Zoologi. Stockholm 30(23): 1–24.

Blanford WT (1881) On a collection of reptiles and frogs chiefly from Singapore. Proceedings of the Zoological Society of London 1881(1): 215–226. https://doi.org/10.1111/j.1096-3642.1881.tb01281.x

Blyth E (1852) Report of Curator, Zoological Department. Journal of the Asiatic Society of Bengal 21: 341–358.

Bordoloi S, Bortamuli T, Ohler A (2007) Systematics of the genus *Rhacophorus* (Amphibia, Anura): identity of red-webbed forms and description of a new species from Assam. Zootaxa 1653: 1–20. https://doi.org/10.11646/zootaxa.1653.1.1

Boulenger GA (1892) An account of the reptiles and batrachians collected by Mr. C. Hose on Mt. Dulit, Borneo. Proceedings of the Zoological Society of London 1892: 505–508.

Boulenger GA (1893) Concluding report on the reptiles and batrachians obtained in Burma by Signor L. Fea dealing with the collection made in Pegu and the Karin Hills in 1887–88. Annali del Museo Civico di Storia Naturale di Genova (Serie 2) 13: 304–347.

Boulenger GA (1908) Descriptions of a new frog and a new snake from Formosa. Annals and Magazine of Natural History (Series 8) 2: 221. https://doi.org/10.1080/00222930808692472

Bourret R (1937) Notes herpétologiques sur l'Indochine française. XIV. Les batraciens de la collection du Laboratoire des Sciences Naturelles de l'Université. Descriptions de quinze especes ou variétés nouvelles. Annexe au Bulletin Général de l'Instruction Publique Hanoi 1937: 5–56.

Brakels P, Nguyen TV, Pawangkhanant P, Idiiatullina S, Suwannapoom C, Poyarkov NA (2023) Mountain jade: A new high–elevation microendemic species of the genus *Zhangixalus* (Amphibia: Anura: Rhacophoridae) from Laos. Zoological Research 44: 374–379. https://doi.org/10.24272/j.issn.2095-8137.2022.382

Chen WC, Liao XW, Zhou SC, Mo YM, Huang Y (2018) Rediscovery of *Rhacophors yaoshanensis* and *Theloderma kwangsiensis* at their type localities after five decades. Zootaxa 4379(4): 484–496. https://doi.org/10.11646/zootaxa.4379.4.2

Chou WH, Lau MW, Chan BPL (2007) A new treefrog of the genus *Rhacophorus* (Anura: Rhacophoridae) from Hainan Island, China. Raffles Bulletin of Zoology Singapore 55: 157–165.

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9: 772. https://doi.org/10.1038/nmeth.2109

David A (1872) [1871] Rapport adressé a MM. les Professeurs–Administráteurs du Museum d'histoire naturelle. Nouvelles Archives du Muséum d'Histoire Naturelle Paris 7: 75–100.

Dufresnes C, Litvinchuk SN (2022) Diversity, distribution and molecular species delimitation in frogs and toads from the Eastern Palaearctic. Zoological Journal of the Linnean Society 195: 695–760. https://doi.org/10.1093/zoolinnean/zlab083

Fei L (1999) Atlas of Amphibians of China., Henan Publishing House of Science and Technology, Zhengzhou.

Fei L, Hu SQ, Ye CY, Huang YZ (2009) Fauna Sinica. Amphibia. Volume 3. Anura Ranidae. Science Press, Beijing.

Fei L, Ye CY, Jiang JP (2010) Colored Atlas of Chinese Amphibians.: Sichuan Publishing House of Science and Technology, Chengdu.

Fei L, Ye YC, Jiang JP (2012) Colored Atlas of Chinese Amphibians and Their Distributions. Sichuan Publishing House of Science and Technology, Chengdu.

Frost DR (2024) Amphibian Species of the World: an Online Reference. Version 6.2. American Museum of Natural History, New York. https://amphibiansoftheworld.amnh.org/index.php [Accessed 15 Oct 2024]

Gonggoli AD, Munir M, Kaprawi F, Kirschey T, Hamidy A (2024) A new species of tree frog (Amphibia, Anura, Rhacophoridae) from Central Java, Indonesia. Raffles Bulletin of Zoology Singapore 72: 219–234. https://doi.org/10.26107/RBZ–2024–0019

Hallowell E (1861) [1860] Report upon the Reptilia of the North Pacific Exploring Expedition, under command of Capt. John Rogers, U.S. N. Proceedings. Academy of Natural Sciences of Philadelphia 12: 480–510.

Harvey MB, Pemberton AJ, Smith EN (2002) New and poorly known parachuting frogs (Rhacophoridae: *Rhacophorus*) from Sumatra and Java. Herpetological Monographs 16: 46–92. https://doi.org/10.1655/0733-1347(2002)016[0046:NAPKPF]2.0.CO;2

He XR (1999) A new species of the family Rhacophoridae from Yunnan—*Polypedates puerensis*. Sichuan Journal of Zoology 18: 99–100.

Hong F, Yu L, Han J, Fang J (2024) Discussion on the Attribution of Species of the Genus *Zhangixalus* at the Junction of Zhejiang and Anhui, China. Chinese Journal of Zoology 59: 225–232. https://doi.org/10.13859/j.cjz.202422288

Hou SB, Yuan ZY, Jiang K, Zhao LS, Wang SN, Wu ZJ, Che J (2017) *Rhacophorus dorsoviridis* Bourret, a new record of amphibians in Guangxi and comparison with morphologically similar species. Sichuan Journal of Zoology 36: 59–60.

Inger RF (1947) Preliminary survey of the amphibians of the Riukiu islands. Fieldiana Zoology 32: 297–352. https://doi.org/10.5962/bhl.title.2991

Inger RF (1966) The systematics and zoogeography of the Amphibia of Borneo. Fieldiana Zoology 52: 1–402. https://doi.org/10.5962/bhl.title.3147

Jiang K, Wang K, Yang JX, Jin J, Dahu Z, Yan F, Pan H, Che J (2016) Two new records of Amphibia from Tibet, China, with description of *Rhacophorus burmanus*. Sichuan Journal of Zoology 35(2): 210–216.

Jiang D, Jiang K, Ren J, Wu J, Li J (2019) Resurrection of the genus *Leptomantis*, with description of a new genus to the family Rhacophoridae (Amphibia: Anura). Asian Herpetological Research 10: 1–12. https://doi.org/10.16373/j.cnki.ahr.180058

Leache AD, Reeder TW (2002) Molecular systematics of the Eastern Fence Lizard (*Sceloporus undulatus*): a comparison of Parsimony, Likelihood, and Bayesian approaches. Systematic Biology 51: 44–68. https://doi.org/10.1080/106351502753475871

Li JT, Li Y, Murphy RW, Rao DQ, Zhang YP (2012a) Phylogenetic resolution and systematics of the Asian tree frogs, *Rhacophorus* (Rhacophoridae, Amphibia). Zoologica Scripta 41: 557–570. https://doi.org/10.1111/j.1463-6409.2012.00557.x

Li JT, Liu J, Chen YY, Wu JW, Murphy RW, Zhao EM, Wang YZ, Zhang YP (2012b) Molecular phylogeny of treefrogs in the *Rhacophorus dugritei* species complex (Anura: Rhacophoridae), with descriptions of two new species. Zoological Journal of the Linnean Society 165: 143–162. https://doi.org/10.1111/j.1096-3642.2011.00790.x

Liang YS, Wang CS (1978) A new tree frog *Rhacophorus taipeianus* (Anura: Rhacophoridae) from Taiwan (Formosa). Quarterly Journal of the Taiwan Museum 31: 185–202.

Liu CC (1945) New frogs from West China. Journal of the West China Border Research Society, Series B 15: 28–44.

Liu CC, Hu SQ (1960) [1959] Preliminary report of Amphibia from southern Yunnan. Acta Zoologica Sinica: 11: 508–538.

Liu CC, Hu SQ (1961) Tailless Amphibians of China. Science Press, Beijing.

Liu CC, Hu SQ (1962) A herpetological report of Kwangsi. Acta Zoologica Sinica, 73–104.

Liu C, Hu S, Yang FH (1962) Preliminary report of Amphibia from western Kweichow. Acta Zoologica Sinica 14: 381–392.

Liu BQ, Wang YF, Jiang K, Chen HM, Zhou JJ, Xu JN, Wu CH. (2017) A new treefrog species of the genus *Rhacophorus* Found in Zhejiang, China (Anura: Rhacophoridae). Chinese Journal of Zoology 52: 361–372.

Lue KY, Lai JS, Chen SL (1994) A new species of *Rhacophorus* (Anura: Rhacophoridae) from Taiwan. Herpetological 50: 303–308.

Lue KY, Lai JS, Chen SL (1995) A new species of *Rhacophorus* (Anura: Rhacophoridae) from Taiwan. Journal of Herpetology 29: 338–345. https://doi.org/10.2307/1564982

Lue BQ, Huang JK, Zhou JQ, Lu WZ, Zhou JJ, Wang YF, Zhou LM, Chen LH (2024) New subspecies of *Zhangixalus* (Amphibia, Anura, Rhacophoridae) —— *Zhangixalus taipeianus jingningensis*. Chinese Journal of Wildlife 45(04): 912–920. https://link.cnki.net/urlid/23.1587.s.20240506.1529.002

Matsui M, Panha S (2006) A new species of *Rhacophorus* from eastern Thailand (Anura: Rhacophoridae). Zoological Science 23(5): 477–481. https://doi.org/10.2108/zsj.23.477

Mo YM, Chen WC, Liao X, Zhou SC (2016) A new species of the genus *Rhacophorus* (Anura: Rhacophoridae) from southern China. Asian Herpetological Research 7: 139–150. https://doi.org/10.16373/j.cnki.ahr.150070

Mou YP, Risch JP, Lue KY (1983) *Rhacophorus prasinatus*, a new tree frog from Taiwan, China (Amphibia, Anura, Rhacophoridae). Alytes 2: 154–162.

Myers CW, Duellman WE (1982) A new species of Hyla from Cerro Colorado, and other tree frog records and geographical notes from western Panama. American Museum Novitates 2752: 1–32.

Nguyen TT, Matsui M, Eto K, Orlov NL (2014) A preliminary study of phylogenetic relationships and taxonomic problems of Vietnamese *Rhacophorus* (Anura: Rhacophoridae). Russian Journal of Herpetology 21(4): 274–280.

Nguyen TT, Ninh HT, Orlov N, Nguyen TQ, Ziegler T (2020) A new species of the genus *Zhangixalus* (Amphibia: Rhacophoridae) from Vietnam. Journal of Natural History 54: 257–273. https://doi.org/10.1080/00222933.2020.1754484

Nguyen TT, Nguyen HH, Ninh HT, Le LTH, Bui HT, Orlov N, Hoang CV, Ziegler T (2024) *Zhangixalus thaoae* sp. nov., a new green treefrog species from Vietnam (Anura, Rhacophoridae). ZooKeys 1197: 93–113. https://doi.org/10.3897/zookeys.1197.104851

Ninh HT, Nguyen TT, Orlov N, Nguyen TQ, Ziegler T (2020) A new species of the genus *Zhangixalus* (Amphibia: Rhacophoridae) from Vietnam. European Journal of Taxonomy 688(688): 1–8. https://doi.org/10.5852/ejt.2020.688

Ohler A, Marquis O, Swan SR, Grosjean S (2000) Amphibian biodiversity of Hoang Lien Nature Reserve (Lao Cai Province, northern Vietnam) with description of two new species. Herpetozoa Wien 13: 71–87.

Okada Y, Kawano U (1924) On the ecological distribution of two new varieties of *Rhacophorus* in Japan. Zoological Magazine 36: 104–109. [144–153]

Orlov NL, Lathrop A, Murphy RW, Ho CT (2001) Frogs of the family Rhacophoridae (Anura: Amphibia) in the northern Hoang Lien Mountains (Mount Fan Si Pan, Sa Pa District, Lao Cai Province), Vietnam. Russian Journal of Herpetology 8: 17–44.

Orlov NL, Nguyen N, Cuc HT (2008) Description of a new species and new records of *Rhacophorus* genus (Amphibia: Anura: Rhacophoridae) with the review of amphibians and reptiles diversity of Ghu Yang Sin National Park (Dac Lac Province, Vietnam). Russian Journal of Herpetology 15: 67–84.

Ostroshabov AA, Orlov NT, Nguyen TT (2013) Taxonomy of Frogs of Genus *Rhacophorus* of "hoanglienensis orlovi" Complex. Russian Journal of Herpetology 20: 301–324.

Pan T, Zhang Y, Wang H, Wu J, Kang X, Qian L, Li K, Zhang Y, Chen J, Rao D, Jiang JP, Zhang B (2017) A New Species of the Genus *Rhacophorus* (Anura: Rhacophoridae) from Dabie Mountains in East China. Asian Herpetological Research 8: 1–13.

Pan YQ, Hou M, Yu GH, Liu S (2024) A new species of *Zhangixalus* (Anura, Rhacophoridae) from Yunnan, China. Zoosystematics and Evolution 100: 183–197. https://doi.org/10.3897/zse.100.113850

Pham AV, Nguyen T, Ziegler T, Nguyen TT (2017) New records of tree frogs (Anura: Rhacophoridae: *Rhacophorus*) from Son La Province, Vietnam. Herpetology Notes 10: 379–386.

Poyarkov NA, Nguyen TV, Popov ES, Geissler P, Pawangkhanant P, Suwannapoom TNC, Orlov NL (2021) Recent progress in taxonomic studies, biogeographic analysis, and revised checklist of amphibians of Indochina. Russian Journal of Herpetology 28 (3A): 1–110. https://doi.org/10.30906/1026-2296-2021-28-3A-1-110

Puillandre N, Brouillet S, Achaz G (2021) ASAP: assemble species by automatic partitioning. Molecular Ecology Resources 21: 609–620. https://doi.org/10.1111/1755-0998.13281

Rao DQ, Wilkinson JA, Liu HN (2006) A new species of *Rhacophorus* (Anura: Rhacophoridae) from Guangxi Province, China. Zootaxa 1258: 17–31. https://doi.org/10.11646/zootaxa.1258.1.2

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Hohna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542. https://doi.org/10.1093/sysbio/sys029

Shui KC, Zhang SY, Liu ZF, Zhu YH, Gong YN, Zhao J (2023) Classification of *Zhangixalus* species in Nanling National Nature Reserve, Guangdong two new provincial records were recorded. Sichuan Journal of Zoology 42: 75–84. https://doi.org/10.11984/j.issn.1000-7083.20220257

Smith MA (1924a) Two lizards and a new tree frog from the Malay Peninsula. Journal of the Federated Malay States Museums 11: 183–186.

Smith MA (1924b) New tree-frogs from Indo-China and the Malay Peninsula. Proceedings of the Zoological Society of London 1924: 225–234. https://doi.org/10.1111/j.1096-3642.1924.tb01499.x

Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post–analysis of large phylogenies. Bioinformatics 30: 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Stejneger L (1907) Herpetology of Japan and adjacent territory. Bulletin of the United States National Museum 58: 1–577. https://doi.org/10.5479/si.03629236.58.i

Stejneger L (1924) Herpetological novelties from China. Occasional Papers of the Boston Society of Natural History 5: 119–121.

Tamura K, Stecher G, Kumar S (2021) MEGA11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution 38: 3022–3027. https://doi.org/10.1093/molbev/msab120

Yu GH, Rao DQ, Zhang MW, Yang JX (2009) Re–examination of the phylogeny of Rhacophoridae (Anura) based on mitochondrial and nuclear DNA. Molecular Phylogenetics and Evolution 50: 571–579. https://doi.org/10.1016/j.ympev.2008.11.023

Yu GH, Hui H, Hou M, Wu ZJ, Rao DQ, Yang JX (2019a) A new species of *Zhangixalus* (Anura: Rhacophoridae), previously confused with *Zhangixalus smaragdinus* (Blyth, 1852). Zootaxa 4711(2): 275–292. https://doi.org/10.11646/zootaxa.4711.2.3

Yu GH, Hong H, Wang J, Rao DQ, Wu ZJ, Yang JX. (2019b). A new species of *Gracixalus* (Anura, Rhacophoridae) from Yunnan, China. ZooKeys 851: 91–111. https://doi.org/10.3897/zookeys.851.32157

Zhang J, Jiang K, Hou M (2011) *Rhacophorus dorsoviridis* Bourret, a new record of family Rhacophoridae to China. Acta Zootaxonomica Sinica 36: 986–989.

Supplementary material 1

Measurements of *Z. daweishanensis* sp. nov., *Z. nanshanensis* sp. nov., *Z. dorsoviridis*, *Z. nigropunctatus*, *Z. lishuiensis*, *Z. yunnanensis* and *Z. zhoukaiyae*

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Supplementary material 2

Mean uncorrected pairwise distances (%) between clades of *Z. daweishanensis* sp. nov. and *Z. nanshanensis* sp. nov. and related species based on 16S rRNA sequences

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Supplementary material 3

Morphological comparison between the new species and congeners of *Zhangixalus*

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Data type: xlsx
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Supplementary material 4

Additional tables and figures

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Data type: docx
Explanation note: **table S4.** Summary statistics of male specimens (mean ± standard deviation) and results of the t-test between the *Z. nanshanensis* sp. nov. (n = 7) and *Z. nigropunctatus* (n = 6). The t-test was performed on the size-adjusted data, except SVL. * = $p < 0.05$, ** = $p < 0.01$. **table S5.** Summary statistics of male specimens (mean ± standard deviation) and results of the t-test between the *Z. nanshanensis* sp. nov. (n = 7) and *Z. yunnanensis* (n = 7). The t-test was performed on the size-adjusted data, except SVL. * = $p < 0.05$, ** = $p < 0.01$. **table S6.** Summary statistics of male specimens (mean ± standard deviation) and results of the t-test between the *Z. lishuiensis* (n = 3) and *Z. zhoukaiyae* (n = 6). The t-test was performed on the size-adjusted data, except SVL. * = $p < 0.05$, ** = $p < 0.01$. **fig. S1.** Views of *Z. zhoukaiyae* (A & a), *Z. nigropunctatus* (B & b; photos by Guohua Yu), *Z. dorsoviridis* (C & c), *Z. lishuiensis* (D & d; photos by Guohua Yu), *Z. nanshanensis* sp. nov. (E & e; photos by Guohua Yu), and *Z. daweishanensis* sp. nov. (F & f; photos by Guohua Yu) in life. A & a were reproduced from Pan et al. (2017), C was reproduced from Mo et al. (2016), and c was reproduced from Zhang et al. (2011).
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